

FORM PTO 1390
(REV 9-2001)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

HO-P02428US0

TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)

APPLICATION NO. (If known, see 37 C.F.R. § 1.102)
10 / 089928 Unknown

INTERNATIONAL APPLICATION NO
PCT/GB00/03800

INTERNATIONAL FILING DATES
October 4, 2000

PRIORITY DATE CLAIMED
October 4, 1999

| | |
|--------------------|------------------------|
| TITLE OF INVENTION | UTROPHIN GENE PROMOTER |
|--------------------|------------------------|

APPLICANT(S) FOR DO/EO/US Edward Burton, et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing 35 U.S.C. 371
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371 (f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c)(2))
 - a. ☒ is attached hereto (required only if not communicated by the International Bureau).
 - b. ☐ has been communicated by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371 (c)(2)).
 - a. ☐ is attached hereto.
 - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
 - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
 - b. ☐ have been communicated by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 11 to 20 below concern document(s) or information included:

- | | | |
|-----|-------------------------------------|---|
| 11. | <input checked="" type="checkbox"/> | An Information Disclosure Statement under 37 CFR 1.97 and 1.98. |
| 12. | <input type="checkbox"/> | An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. |
| 13. | <input checked="" type="checkbox"/> | A FIRST preliminary amendment. |
| 14. | <input type="checkbox"/> | A SECOND or SUBSEQUENT preliminary amendment. |
| 15. | <input type="checkbox"/> | A substitute specification. |
| 16. | <input type="checkbox"/> | A change of power of attorney and/or address letter. |
| 17. | <input checked="" type="checkbox"/> | A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825. |
| 18. | <input type="checkbox"/> | A second copy of the published international application under 35 U.S.C. 154(d)(4). |
| 19. | <input type="checkbox"/> | A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4). |
| 20. | <input type="checkbox"/> | Other items or information: SEE ATTACHED RETURN POSTCARD |

107089928
Unknown

JC13 Rec'd PCT/PTO 04 APR 2002

| U.S. APPLICATION NO. (if known, see 37 CFR 1.51) 107089928 | | INTERNATIONAL APPLICATION NO. PCT/GB00/03800 | | ATTORNEY'S DOCKET NUMBER HO-P02428US0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| 21. <input checked="" type="checkbox"/> The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) – (5)): <input type="checkbox"/> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$1040.00 <input checked="" type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$890.00 <input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$740.00 <input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$710.00 <input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) \$100.00 ENTER APPROPRIATE BASIC FEE AMOUNT = Surcharge of \$ <u>130.00</u> for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input checked="" type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (e)). | | | | CALCULATIONS PTO USE ONLY <table style="width:100%; border-collapse: collapse;"> <tr><td style="width: 10%; text-align: right;">\$</td><td style="width: 80%;">890.00</td><td style="width: 10%;"></td></tr> <tr><td style="text-align: right;">\$</td><td>130.00</td><td></td></tr> </table> | | \$ | 890.00 | | \$ | 130.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| \$ | 890.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| \$ | 130.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <table border="1" style="width:100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 20%;">CLAIMS</th> <th style="width: 20%;">NUMBER FILED</th> <th style="width: 20%;">NUMBER EXTRA</th> <th style="width: 20%;">RATE</th> </tr> </thead> <tbody> <tr> <td>Total claims</td> <td>113-20</td> <td>93</td> <td>x 18.00</td> </tr> <tr> <td>Independent claims</td> <td>15-3</td> <td>12</td> <td>x 84.00</td> </tr> <tr> <td colspan="3">MULTIPLE DEPENDENT CLAIM(s) (if applicable)</td> <td>+ 280.00</td> </tr> <tr> <td colspan="3">TOTAL OF ABOVE CALCULATIONS =</td> <td></td> </tr> </tbody> </table> | | CLAIMS | NUMBER FILED | NUMBER EXTRA | RATE | Total claims | 113-20 | 93 | x 18.00 | Independent claims | 15-3 | 12 | x 84.00 | MULTIPLE DEPENDENT CLAIM(s) (if applicable) | | | + 280.00 | TOTAL OF ABOVE CALCULATIONS = | | | | <table border="1" style="width:100%; border-collapse: collapse;"> <tbody> <tr><td style="width: 10%; text-align: right;">\$</td><td style="width: 80%;">1,674.00</td><td style="width: 10%;"></td></tr> <tr><td style="text-align: right;">\$</td><td>1,008.00</td><td></td></tr> <tr><td style="text-align: right;">\$</td><td>280.00</td><td></td></tr> <tr><td style="text-align: right;">\$</td><td>3,982.00</td><td></td></tr> <tr><td colspan="3">SUBTOTAL =</td></tr> <tr><td style="text-align: right;">\$</td><td>3,982.00</td><td></td></tr> <tr><td colspan="3">Processing fee of \$ _____ for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +</td></tr> <tr><td style="text-align: right;">\$</td><td></td><td></td></tr> <tr><td colspan="3">TOTAL NATIONAL FEE =</td></tr> <tr><td style="text-align: right;">\$</td><td>3,982.00</td><td></td></tr> <tr><td colspan="3">Fee for recording the enclosed assignment (37 CFR 1.21 (h)). Assignment must be accompanied by appropriate cover sheet (37 CFR 3.28, 3.31) (_____ per property). +</td></tr> <tr><td style="text-align: right;">\$</td><td></td><td></td></tr> <tr><td colspan="3">TOTAL FEES ENCLOSED =</td></tr> <tr><td style="text-align: right;">\$</td><td>3,982.00</td><td></td></tr> <tr><td colspan="3">Amount to be Refunded:</td></tr> <tr><td style="text-align: right;">\$</td><td></td><td></td></tr> <tr><td colspan="3">Charged:</td></tr> <tr><td style="text-align: right;">\$</td><td></td><td></td></tr> </tbody> </table> | | \$ | 1,674.00 | | \$ | 1,008.00 | | \$ | 280.00 | | \$ | 3,982.00 | | SUBTOTAL = | | | \$ | 3,982.00 | | Processing fee of \$ _____ for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). + | | | \$ | | | TOTAL NATIONAL FEE = | | | \$ | 3,982.00 | | Fee for recording the enclosed assignment (37 CFR 1.21 (h)). Assignment must be accompanied by appropriate cover sheet (37 CFR 3.28, 3.31) (_____ per property). + | | | \$ | | | TOTAL FEES ENCLOSED = | | | \$ | 3,982.00 | | Amount to be Refunded: | | | \$ | | | Charged: | | | \$ | | |
| CLAIMS | NUMBER FILED | NUMBER EXTRA | RATE | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Total claims | 113-20 | 93 | x 18.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Independent claims | 15-3 | 12 | x 84.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| MULTIPLE DEPENDENT CLAIM(s) (if applicable) | | | + 280.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL OF ABOVE CALCULATIONS = | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| \$ | 1,674.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| \$ | 1,008.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| \$ | 280.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| Processing fee of \$ _____ for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). + | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| Amount to be Refunded: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| \$ | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Charged: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| \$ | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| a. <input checked="" type="checkbox"/> A check in the amount of \$ <u>3,982.00</u> to cover the above fees is enclosed. b. <input type="checkbox"/> Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed. c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required or credit any overpayment to my Deposit Account No. <u>06-2375</u> . A duplicate copy of this sheet is enclosed. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SEND ALL CORRESPONDENCE TO: Melissa W. Acosta FULBRIGHT & JAWORSKI L.L.P. 1301 McKinney, Suite 5100 Houston, Texas 77010-3095 (713) 651-5407 | | | SIGNATURE: NAME: _____ REGISTRATION NUMBER: <u>45,872</u> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

10/089928

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as Express Mail, Airbill No. EU098493497US, in an envelope addressed to: Commissioner for Patents, Washington, DC 20231, on the date shown below.

Dated: April 4, 2002

Signature:

Staci Harris
(Staci Harris)

JC13 Rec'd PCT/PTC 0 4 APR 2002

Docket No.: HO-P02428US0
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Edward Burton, et al.

Application No.: Unknown

Group Art Unit:

Filed: April 4, 2002

Examiner:

For: UTROPHIN GENE PROMOTER

STATEMENT PURSUANT TO 37 CFR 1.823(b)

U.S. Patent and Trademark Office
Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Submitted herewith for filing in connection with the above-referenced patent application is a labeled, computer readable copy of the Sequence Listing included in the application.

I hereby state that I have reviewed the paper copy of the Sequence Listing contained, as required by 37 CFR 1.821(e), and the computer readable form of the Sequence Listing, as required by 37 CFR 1.821(c), and that the content of the paper and computer readable copies are the same.

Early favorable consideration of the patent application is respectfully solicited.

Dated: April 4, 2002

Respectfully submitted,

By

Melissa W. Acosta
Melissa W. Acosta

Registration No.: 45,872
FULBRIGHT & JAWORSKI L.L.P.
1301 McKinney, Suite 5100
Houston, Texas 77010-3095
(713) 651-5407
(713) 651-5246 (Fax)

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as Express Mail, Airbill No. EU098493497US, in an envelope addressed to: Box Non-Fee Amendment, Commissioner for Patents, Washington, DC 20231, on the date shown below.

Dated: April 4, 2002

Signature: 
(Melissa W. Acosta)

Docket No.: HO-P02428US0

(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Edward Burton, et al.

Application No.: UNKNOWN

Group Art Unit: N/A

Filed: April 4, 2002

Examiner: Not Yet Assigned

For: UTROPHIN GENE PROMOTER

FIRST PRELIMINARY AMENDMENT

Box Non-Fee Amendment

Commissioner for Patents
Washington, DC 20231

Dear Sir:

Prior to examination on the merits, please amend the above-identified U.S. patent application as follows:

In the Claims

Please add the following new claims 53-111.

53. An isolated nucleic acid comprising a promoter which comprises a sequence of nucleotides selected from (i) the human promoter sequence shown in Figure 1 and (ii) the mouse promoter sequence shown in Figure 2, free or substantially free of utrophin coding sequence.

54. An isolated nucleic acid consisting essentially of a promoter which comprises the sequence of nucleotides shown 5' to position 1440 in Figure 1.

55. An isolated nucleic acid consisting essentially of a promoter which comprises the sequence of nucleotides shown 5' to position 1183 of the mouse sequence shown in Figure 2.

56. An isolated nucleic acid consisting essentially of a promoter which comprises the nucleotides numbered 1199 -1440 in the sequence shown in Figure 1.

57. An isolated nucleic acid consisting essentially of a promoter which comprises the nucleotides numbered 959-1183 in the sequence shown in Figure 2.

58. An isolated nucleic acid consisting essentially of a promoter which comprises the nucleotide sequence ACAGGACATCCCAGTGTGCAGTTCG.

59. An isolated nucleic acid consisting essentially of a promoter which comprises a sequence of nucleotides that is an allele, mutant or derivative, by way of addition, insertion, deletion or substitution of one or more nucleotides, of the promoter sequence shown in Figure 1, which sequence has at least 60% homology with the promoter sequence shown in figure 1 and which promoter, when operably linked to a sequence of nucleotides, has the ability to initiate transcription of that sequence, said transcription being muscle-specific.

60. An isolated nucleic acid consisting essentially of a promoter which comprises a sequence of nucleotides that is an allele, mutant or derivative, by way of addition, insertion, deletion or substitution of one or more nucleotides, of the promoter sequence shown in Figure 2, which sequence has at least 60% homology with the promoter sequence shown in figure 2 and which promoter, when operably linked to a sequence of nucleotides, has the ability to initiate transcription of that sequence, said transcription being muscle-specific.

61. An isolated nucleic acid consisting essentially of a promoter which comprises a sequence of nucleotides that is an allele, mutant or derivative, by way of addition, insertion, deletion or substitution of one or more nucleotides, of the promoter sequence shown in Figure 2, which hybridises to the promoter sequence shown in figure 2 under stringent hybridisation conditions and which promoter, when operably linked to a sequence of nucleotides, has the ability to initiate transcription of that sequence, said transcription being muscle-specific.

62. A nucleic acid construct comprising an isolated nucleic acid according to any of the preceding claims operably linked to a heterologous sequence.

63. A nucleic acid construct comprising an isolated nucleic acid according to any one of claims 53 to 61 operably-linked to a coding sequence.

64. A nucleic acid construct according to claim 63 wherein said coding sequence encodes a reporter molecule.

65. An in vitro host cell comprising a nucleic acid construct according to claim 63.

66. An in vitro host cell comprising a nucleic acid construct according to claim 64.

67. A method comprising culturing a host cell according to claim 65 under conditions for expression of the peptide or polypeptide encoded by said coding sequence.

68. A method as claimed in claim 67 wherein said coding sequence encodes a reporter molecule.

69. A method according to claim 67 comprising detection of transcription of said coding sequence.

70. A method according to claim 67 comprising detection of expression of the peptide or polypeptide encoded by said coding sequence.

71. A method of screening for a substance able to modulate utrophin promoter activity, the method comprising contacting an expression system containing a nucleic acid construct according to claim 63 with a test or candidate substance and determining transcription of said coding sequence or expression of the peptide or polypeptide encoded by said coding sequence.

72. A method as claimed in claim 63 wherein said coding sequence encodes a reporter molecule and said reporter molecule is detected.

73. A method according to claim 71 wherein the expression system comprises a host cell containing said nucleic acid construct.

74. A method which comprises, following identification of a substance able to modulate utrophin promoter activity in accordance with a method according to claim 71, manufacture of the substance and/or use of the substance in manufacture or formulation of a composition.

75. The use of an isolated nucleic acid according to any of claims 53 to 58 for promoting transcription of an operably linked sequence of nucleotides.

76. The use of claim 75 wherein the transcription is tissue-specific, with the tissue-specificity being muscle-specific.

77. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide including the amino acid sequence shown in Figure 1 or Figure 2.

78. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide that is an allele, mutant or derivative of a polypeptide including the amino acid sequence shown in Figure 1, which amino acid sequence has at least 60% homology with the polypeptide sequence in Figure 1 or Figure 2.

79. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide that is an allele, mutant or derivative of a polypeptide shown in Figure 1 or Figure 2, which nucleotide sequence hybridises with the nucleotide sequence encoding the polypeptide in Figure 1 or Figure 2 under stringent hybridisation conditions.

80. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide having the amino acid sequence shown in Figure 9.

81. An isolated nucleic acid molecule comprising the nucleotide sequence shown in Figure 9.

82. A nucleic acid of any one of claims 77 to 81 comprised in a vector.

83. A nucleic acid according to any one of claims 77 to 81 comprised in an expression vector.

84. An in vitro host cell containing an expression vector according to claim 83.

85. A method including introduction of nucleic acid according to any of claims 77 to 81 into a cell.

86. A method as claimed in claim 85 wherein said nucleic acid is an expression vector.

87. A method according to claim 85 wherein said introduction takes place in vitro.
88. A method as claimed in claim 85 which includes causing or allowing expression of said polypeptide encoding nucleotide sequence in a cell.
89. A method according to claim 88 wherein the cell is part of a mammal.
90. A method according to claim 88 wherein the expression product is purified and/or isolated following expression.
91. A method according to claim 90 wherein the expression product is formulated into a composition which includes at least one additional component, following purification and/or isolation of the expression product.
92. An isolated polypeptide as encoded by nucleic acid according to any of claims 77 to 81.
93. An isolated utrophin exon IB polypeptide selected from:
(i) human utrophin exon IB polypeptide of which the amino acid sequence is shown in Figure 1; and
(ii) mouse utrophin exon IB of which the amino acid sequence is shown in Figure 1.
94. An isolated polypeptide including the human polypeptide according to claim 93.
95. An isolated polypeptide including the mouse polypeptide according to claim 93.
96. An isolated polypeptide which has 60 % homology with the polypeptide according to claim 94 or 95.
97. An isolated fragment of a polypeptide according to claim 93, which fragment is 5 to 25 amino acids in length.
98. An isolated fragment of a polypeptide according to claim 93, which fragment is 10 to 20 amino acids in length.

99. An antibody specific for a polypeptide according to any one of claims 92 to 96.

100. A composition including a polypeptide according to claim 92 and a pharmaceutically acceptable excipient.

101. A composition including a polypeptide according to any one of claims 92 to 98 and a pharmaceutically acceptable excipient.

102. A composition including a polypeptide according to claim 94 and a pharmaceutically acceptable excipient.

103. A composition including a fragment according to claim 97 or claim 98 and a pharmaceutically acceptable excipients.

104. A composition including an antibody according to claim 99 and a pharmaceutically acceptable excipients.

105. A method for treating a dystrophin phenotype in a mammal, which comprises administering a nucleic acid according to any one of claims 77 to 81 in a therapeutically effective amount.

106. A method as claimed in claim 105 wherein said nucleic acid is an expression vector.

107. A method for treating a dystrophin phenotype in a mammal, which comprises administering a polypeptide according to claim 92 in a therapeutically effective amount.

108. A method for treating a dystrophin phenotype in a mammal, which comprises administering a polypeptide according to any one of claims 93 to 95 in a therapeutically effective amount.

109. A method for treating a dystrophin phenotype in a mammal, which comprises administering a polypeptide according to claim 96 in a therapeutically effective amount.

110. A method for treating a dystrophin phenotype in a mammal, which comprises administering a fragment according to claim 97 or claim 98 in a therapeutically effective amount.

111. A method for treating a dystrophin phenotype in a mammal, which comprises administering an antibody according to claim 99 in a therapeutically effective amount.

REMARKS/ARGUMENTS

Claims 1-52 were in the original PCT application as filed. Applicants have canceled claims 53-111, without prejudice or acquiescence and have added claims 53-111. Claims 53-111 delete the multiple dependency and clarify the claims without prejudice or acquiescence. Applicants assert that no new matter has been added.

CONCLUSION

Applicants have added claims 53-111 to delete the multiple dependency and to clarify the claims without prejudice or acquiescence. Claims 53-111 have been canceled without prejudice or acquiescence. Therefore, these amendments do not narrow the scope of the claims within the meaning of *Festo Corp. v. Shoketsu Kinzoku Kogyo Kabushiki Co., Ltd.*, 234 F.3d 558, 586, 56 USPQ2d 1865, 1886 (Fed. Cir. 2000).

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to pass this application to issue.

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UTROPHIN GENE PROMOTER

The present invention is based on cloning of a genomic promoter region of the human utrophin gene and of the mouse utrophin gene.

5 The severe muscle wasting disorders Duchenne muscular dystrophy (DMD) and the less debilitating Becker muscular dystrophy (BMD) are due to mutations in the dystrophin gene resulting in a lack of dystrophin or abnormal expression of truncated forms of dystrophin, respectively. Dystrophin is a
10 large cytoskeletal protein (427kDa with a length of 125nm) which in muscle is located at the cytoplasmic surface of the sarcolemma, the neuromuscular junction (NMJ) and myotendinous junction (MTJ). It binds to a complex of proteins and glycoproteins spanning the sarcolemma called the dystrophin
15 associated glycoprotein complex (DGC). The breakdown of the integrity of this complex due to loss of, or impairment of dystrophin function, leads to muscle degeneration and the DMD phenotype.

The dystrophin gene is the largest gene so far identified in
20 man, covering over 2.7 megabases and containing 79 exons. The corresponding 14kb dystrophin mRNA is expressed predominantly in skeletal, cardiac and smooth muscle with lower levels in brain. Transcription of dystrophin in different tissues is regulated from either the brain promoter (predominantly active
25 in neuronal cells) or muscle promoter (differentiated myogenic cells, and primary glial cells) giving rise to differing first exons. A third promoter between the muscle promoter and the second exon of dystrophin regulates expression in cerebellar Purkinje neurons. Recently reviewed in (Tinsley, et al (1994)
30 *Proc Natl Acad Sci U S A* 91, 8307-13, Blake, et al (1994) *Trends in Cell Biol.* 4: 19-23, Tinsley, et al (1993) *Curr Opin Genet Dev.* 3: 484-90).

There are various approaches which have been adopted for the gene therapy of DMD, using the *mdx* mouse as a model system. However, there are considerable problems related to the number of muscle cells that can be made dystrophin positive, the levels of expression of the gene and the duration of expression (Partridge, et al. (1995) *British Medical Bulletin* 51: 123-137). It has also become apparent that simply re-introducing genes expressing the dystrophin carboxy-terminus has no effect on the dystrophic phenotype although the DGC appears to be re-established at the sarcolemma (Cox, et al. (1994) *Nature Genet* 8: 333-339, Greenberg, et al. (1994) *Nature Genet* 8: 340-344).

In order to circumvent some of these problems, possibilities of compensating for dystrophin loss using a related protein, utrophin, are being explored as an alternative route to dystrophin gene therapy. A similar strategy is currently being evaluated in clinical trials to up-regulate foetal haemoglobin to compensate for the affected adult-globin chains in patients with sickle cell anaemia (Rodgers, et al. (1993) *N Engl J Med.* 328: 73-80, Perrine, et al. (1993) *N Engl J Med.* 328: 81-86).

Utrophin is a 395kDa protein encoded by multiexonic 1Mb *UTRN* gene located on chromosome 6q24 (Pearce, et al. (1993) *Hum Mol Gene.* 2: 1765-1772). At present the tissue regulation of utrophin is not fully understood. In the dystrophin deficient *mdx* mouse, utrophin levels in muscle remain elevated soon after birth compared with normal mice; once the utrophin levels have decreased to the adult levels (about 1 week after birth), the first signs of muscle fibre necrosis are detected. However there is evidence to suggest that in the small calibre muscles, continual increased levels of utrophin can interact with the DGC complex (or an antigenically related complex) at the sarcolemma thus preventing loss of the complex with the result that these muscles appear normal. There is also a

substantial body of evidence demonstrating that utrophin is capable of localising to the sarcolemma in normal muscle. During fetal muscle development there is increased utrophin expression, localised to the sarcolemma, up until 18 weeks in the human and 20 days gestation in the mouse. After this time the utrophin sarcolemmal staining steadily decreases to the significantly lower adult levels shortly before birth where utrophin is localised almost exclusively to the NMJ. The decrease in utrophin expression coincides with increased expression of dystrophin. See reviews (Ibraghimov Beskrovnaya, et al. (1992) *Nature* 355, 696-702., Blake, et al. (1994) *Trends in Cell Biol.* 4: 19-23, Tinsley, et al. (1993) *Curr Opin Genet Dev.* 3: 484-90).

Thus, in certain circumstances utrophin can localise to the sarcolemma probably at the same binding sites as dystrophin, through interactions with actin and the DGC. Accordingly, if expression of utrophin is sufficiently elevated, it may maintain the DGC and thus alleviate muscle degeneration in DMD/BMD patients (Tinsley, et al. (1993) *Neuromuscul Disord* 3, 537-9.).

However, manipulation of utrophin expression and screening for molecules able to upregulate expression is hampered by the limited understanding of utrophin expression regulation and its promoters. We have previously isolated a promoter element lying within the CpG island at the 5' end of the utrophin locus that is active in a broad range of cell types and tissues, and shown it to be synaptically regulated *in vivo* (Dennis, et al. (1996) *Nucleic Acids Res* 24, 1646-52 and WO 96/34101). The sequence contains a consensus N-box, a 6bp motif important in the regulation of other genes expressed at the NMJ (Koike, et al. (1995) *Proc Natl Acad Sci USA* 92, 10624-10628). Localisation of utrophin at the NMJ in mature muscle is partially attributable to enhanced transcription of utrophin at sub-junctional myonuclei, with consequent synaptic

accumulation of mRNA (Gramolini, et al. (1997) *J Biol Chem* 272, 8117-20, Vater, et al. (1998) *Molecular and Cellular Neuroscience* 10, 229-242). The utrophin promoter drives synaptic transcription of a reporter gene in vivo; this expression pattern is abolished by point mutations within the N-box (Gramolin, et al. (1998) *J Biol Chem* 273, 736-43).

The present inventors hypothesised that utrophin might be transcribed from more than one promoter, an important consideration for the following reasons: First, it may be undesirable to interfere with the mechanisms underlying synaptic regulation of genes, as this might affect expression of other post-synaptic components and impair the structure and function of the NMJ; a promoter without synaptic regulatory elements might be a more suitable target for pharmacological manipulation. Second, cardiac dysfunction is a common feature of the dystrophinopathies (Hoogerwaard, et al. (1997) *J Neurol* 244, 657-63, Sasaki, et al. (1998) *Am Heart J* 135, 937-44); if the cardiac utrophin message was transcribed from a different promoter, then it might prove necessary to up-regulate this. Finally, inclusion of additional regulatory sequences might increase the yield of a screening program to identify small molecules capable of transcriptional activation of utrophin.

We have now identified an alternative promoter lying within the large second intron of the utrophin gene, 50kb 3' to exon 2. The promoter is highly regulated, expressed in a wide range of tissues and has little similarity to the synaptically expressed promoter. This promoter drives transcription of a widely expressed unique first exon that splices into a common full-length mRNA at exon 3. This unique exon (called exon IB) encodes a novel 31 amino acid N-terminus for the utrophin protein which may be involved in binding to the muscle membrane. The sequences of the two utrophin promoters are dissimilar, and we predict that they respond to discrete sets of cellular signals.

Exon IB is primarily considered herein to encode the indicated 31 amino acids. However, the splice occurs within a codon for aspartate. This aspartate residue is common to both isoforms of utrophin. In embodiments of the invention an aspartate
5 residue may be included C-terminal to the 31 amino acids to provide a 32 amino acid peptide, which may be joined to additional amino acids, for instance additional utrophin sequence as discussed. See, for instance, Figure 8 for one embodiment.

10 These findings significantly contribute to the understanding of the molecular physiology of utrophin expression and are important because the promoter reported here provides an alternative target for transcriptional activation of utrophin in DMD muscle. This promoter does not contain synaptic
15 regulatory elements and might, therefore, be a more suitable target for pharmacological manipulation than the previously described promoter.

We have now cloned this alternative utrophin promoter and exon, and the present invention in various aspects and
20 embodiments is based on the sequence information obtained and provided herein.

One major use of the promoter is in screening for substances able to modulate its activity. It is well known that pharmaceutical research leading to the identification of a new
25 drug generally involves the screening of very large numbers of candidate substances, both before and even after a lead compound has been found. This is one factor which makes pharmaceutical research very expensive and time-consuming. A method or means assisting in the screening process will have
30 considerable commercial importance and utility. Substances identified as upregulators of the utrophin promoter represent an advance in the fight against muscular dystrophy since they provide basis for design and investigation of therapeutics for

in vivo use.

In one aspect, the present invention provides an isolated nucleic acid comprising a promoter, the promoter comprising a sequence of nucleotides shown in Figure 1 or Figure 2. The promoter may comprise one or more fragments of the sequence shown in Figure 1 of Figure 2 sufficient to promote gene expression. The promoter may comprise or consist essentially of a sequence of nucleotides 5' to position 1440 in Figure 1 (human) or position 1183 in Figure 2 (mouse). Preferably the promoter comprises or consists essentially of nucleotides 1199 to 1440 of the human sequence shown in Figure 1, or the equivalent sequence in mouse, e.g. nucleotides 959 to 1183 of Figure 2.

An even smaller portion of this part of the sequences shown in Figure 1 of Figure 2 may be used as long as promoter activity is retained. Restriction enzymes or nucleases may be used to digest the nucleic acid, followed by an appropriate assay (for example as illustrated herein using luciferase constructs) to determine the minimal sequence required. A preferred embodiment of the present invention provides a nucleic acid isolate with the minimal nucleotide sequence shown in Figure 1 or Figure 2 required for promoter activity. The minimal promoter element is situated between the PvuII restriction site at position 1199 in the human sequence and the transcription start site at 1440 bp in the human sequence and between nucleotides 959 to 1183 in the mouse sequence (see Figure 2).

In one embodiment a promoter according to the present invention comprises or consists of sequence that is shown in Figure 3 to be conserved between the human and mouse sequences, e.g. the 25 nucleotide sequence:
ACAGGACATCCCAGTGTGCAGTTCG spanning the transcriptional start site.

The promoter may comprise one or more sequence motifs or elements conferring developmental and/or tissue-specific regulatory control of expression. For instance, the promoter may comprise a sequence for muscle-specific expression, e.g.
5 an E-box element/myoD binding site, such as CANNTG, preferably CAGGTG.

Other regulatory sequences may be included, for instance as identified by mutation or digest assay in an appropriate expression system or by sequence comparison with available
10 information, e.g. using a computer to search on-line databases.

By "promoter" is meant a sequence of nucleotides from which transcription may be initiated of DNA operably linked downstream (i.e. in the 3' direction on the sense strand of
15 double-stranded DNA).

"Operably linked" means joined as part of the same nucleic acid molecule, suitably positioned and oriented for transcription to be initiated from the promoter. DNA operably linked to a promoter is "under transcriptional initiation
20 regulation" of the promoter.

The present invention extends to a promoter which has a nucleotide sequence which is allele, mutant, variant or derivative, by way of nucleotide addition, insertion, substitution or deletion of a promoter sequence as provided
25 herein. Systematic or random mutagenesis of nucleic acid to make an alteration to the nucleotide sequence may be performed using any technique known to those skilled in the art. One or more alterations to a promoter sequence according to the present invention may increase or decrease promoter activity,
30 or increase or decrease the magnitude of the effect of a substance able to modulate the promoter activity.

"Promoter activity" is used to refer to ability to initiate transcription. The level of promoter activity is quantifiable for instance by assessment of the amount of mRNA produced by transcription from the promoter or by assessment of the amount of protein product produced by translation of mRNA produced by transcription from the promoter. The amount of a specific mRNA present in an expression system may be determined for example using specific oligonucleotides which are able to hybridise with the mRNA and which are labelled or may be used in a specific amplification reaction such as the polymerase chain reaction. Use of a reporter gene as discussed further below facilitates determination of promoter activity by reference to protein production.

In various embodiments of the present invention a promoter which has a sequence that is a fragment, mutant, allele, derivative or variant, by way of addition, insertion, deletion or substitution of one or more nucleotides, of the sequence of either the human or the mouse promoters shown in Figures 1 and 2, respectively, has at least about 60% homology with one or both of the shown sequences, preferably at least about 70% homology, more preferably at least about 80% homology, more preferably at least about 90% homology, more preferably at least about 95% homology. The sequence in accordance with an embodiment of the invention may hybridise with one or both of the shown sequences, or the complementary sequences (since DNA is generally double-stranded).

Similarity or homology (the terms are used interchangeably) or identity is preferably determined using GAP, from version 20 of GCG. This uses the algorithm of Needleman and Wunsch to align sequences inserting gaps as appropriate to improve the agreement between the two sequences. Parameters employed are the default ones: for nucleotide sequences - Gap Weight 50, Length Weight 3, Average Match 10.000, Average Mismatch 0.000; for peptide sequences - Gap Weight 8, Length Weight 2, Average

Match 2.912, Average Mismatch -2.003. Peptide similarity scores are taken from the BLOSUM62 matrix. Also useful is the TBLASTN program, of Altschul et al. (1990) *J. Mol. Biol.* 215: 403-10, or BestFit, which is part of the Wisconsin Package, Version 8, September 1994, (Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA, Wisconsin 53711). Sequence comparisons may be made using FASTA and FASTP (see Pearson & Lipman, 1988. *Methods in Enzymology* 183: 63-98). Parameters are preferably set, using the default matrix, as follows: Gapopen (penalty for the first residue in a gap): -12 for proteins / -16 for DNA; Gapext (penalty for additional residues in a gap): -2 for proteins / -4 for DNA; KTUP word length: 2 for proteins / 6 for DNA.

Nucleic acid sequence homology may be determined by means of selective hybridisation between molecules under stringent conditions.

Preliminary experiments may be performed by hybridising under low stringency conditions. For probing, preferred conditions are those which are stringent enough for there to be a simple pattern with a small number of hybridisations identified as positive which can be investigated further.

For example, hybridizations may be performed, according to the method of Sambrook et al. (below) using a hybridization solution comprising: 5X SSC (wherein 'SSC' = 0.15 M sodium chloride; 0.15 M sodium citrate; pH 7), 5X Denhardt's reagent, 0.5-1.0% SDS, 100 µg/ml denatured, fragmented salmon sperm DNA, 0.05% sodium pyrophosphate and up to 50% formamide. Hybridization is carried out at 37-42°C for at least six hours. Following hybridization, filters are washed as follows: (1) 5 minutes at room temperature in 2X SSC and 1% SDS; (2) 15 minutes at room temperature in 2X SSC and 0.1% SDS; (3) 30 minutes - 1 hour at 37°C in 1X SSC and 1% SDS; (4) 2 hours at 42-65°C in 1X SSC and 1% SDS, changing the solution

every 30 minutes.

One common formula for calculating the stringency conditions required to achieve hybridization between nucleic acid molecules of a specified sequence homology is (Sambrook et al., 1989): $T_m = 81.5^{\circ}\text{C} + 16.6\text{Log} [\text{Na}^+] + 0.41 (\% \text{ G+C}) - 0.63 (\% \text{ formamide}) - 600/\text{\#bp in duplex}.$

As an illustration of the above formula, using $[\text{Na}^+] = [0.368]$ and 50-% formamide, with GC content of 42% and an average probe size of 200 bases, the T_m is 57°C . The T_m of a DNA duplex decreases by 1 - 1.5°C with every 1% decrease in homology. Thus, targets with greater than about 75% sequence identity would be observed using a hybridization temperature of 42°C . Such a sequence would be considered substantially homologous to the nucleic acid sequence of the present invention.

It is well known in the art to increase stringency of hybridisation gradually until only a few positive clones remain. Other suitable conditions include, e.g. for detection of sequences that are about 80-90% identical, hybridization overnight at 42°C in 0.25M Na_2HPO_4 , pH 7.2, 6.5% SDS, 10% dextran sulfate and a final wash at 55°C in 0.1X SSC, 0.1% SDS. For detection of sequences that are greater than about 90% identical, suitable conditions include hybridization overnight at 65°C in 0.25M Na_2HPO_4 , pH 7.2, 6.5% SDS, 10% dextran sulfate and a final wash at 60°C in 0.1X SSC, 0.1% SDS.

In a further embodiment, hybridisation of nucleic acid molecule to an allele or variant may be determined or identified indirectly, e.g. using a nucleic acid amplification reaction, particularly the polymerase chain reaction (PCR). PCR requires the use of two primers to specifically amplify target nucleic acid, so preferably two nucleic acid molecules

with sequences characteristic of the utrophin promoter are employed. Using RACE PCR, only one such primer may be needed (see "PCR protocols; A Guide to Methods and Applications", Eds. Innis *et al*, Academic Press, New York, (1990)).

5 Thus a method involving use of PCR in obtaining nucleic acid according to the present invention may include:

(a) providing a preparation of nucleic acid, e.g. from a muscle cell;

10 (b) providing a pair of nucleic acid molecule primers useful in (i.e. suitable for) PCR, at least one of said primers being a primer specific for nucleic acid according to the present invention;

(c) contacting nucleic acid in said preparation with said primers under conditions for performance of PCR;

15 (d) performing PCR and determining the presence or absence of an amplified PCR product.

The presence of an amplified PCR product may indicate identification of an allele or other variant. The sequence may have the ability to promote transcription (i.e. have
20 "promoter activity") in muscle cells, e.g. human muscle cells, or muscle-specific transcription.

Further provided by the present invention is a nucleic acid construct comprising a utrophin promoter region or a fragment, mutant, allele, derivative or variant thereof able to promoter
25 transcription, operably linked to a heterologous gene, e.g. a coding sequence. By "heterologous" is meant a gene other than utrophin. Modified forms of utrophin are generally excluded. Generally, the gene may be transcribed into mRNA which may be translated into a peptide or polypeptide product which may be
30 detected and preferably quantitated following expression. A gene whose encoded product may be assayed following expression is termed a "reporter gene", i.e. a gene which "reports" on promoter activity.

The reporter gene preferably encodes an enzyme which catalyses a reaction which produces a detectable signal, preferably a visually detectable signal, such as a coloured product. Many examples are known, including β -galactosidase and luciferase.

5 β -galactosidase activity may be assayed by production of blue colour on substrate, the assay being by eye or by use of a spectrophotometer to measure absorbance. Fluorescence, for example that produced as a result of luciferase activity, may be quantitated using a spectrophotometer. Radioactive assays
10 may be used, for instance using chloramphenicol acetyltransferase, which may also be used in non-radioactive assays. The presence and/or amount of gene product resulting from expression from the reporter gene may be determined using a molecule able to bind the product, such as an antibody or
15 fragment thereof. The binding molecule may be labelled directly or indirectly using any standard technique.

Those skilled in the art are well aware of a multitude of possible reporter genes and assay techniques which may be used to determine gene activity. Any suitable reporter/assay may
20 be used and it should be appreciated that no particular choice is essential to or a limitation of the present invention.

Expression of a reporter gene from the promoter may be in an *in vitro* expression system or may be intracellular (*in vivo*). Expression generally requires the presence, in addition to the
25 promoter which initiates transcription, a translational initiation region and transcriptional and translational termination regions. One or more introns may be present in the gene, along with mRNA processing signals (e.g. splice sites).

30 Systems for cloning and expression of a polypeptide are discussed further below.

The present invention also provides a nucleic acid vector

comprising a promoter as disclosed herein. Such a vector may comprise a suitably positioned restriction site or other means for insertion into the vector of a sequence heterologous to the promoter to be operably linked thereto.

- 5 Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator fragments, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. For further details see, for example, *Molecular*
10 *Cloning: a Laboratory Manual*: 2nd edition, Sambrook et al, 1989, Cold Spring Harbor Laboratory Press. Procedures for introducing DNA into cells depend on the host used, but are well known.

Thus, a further aspect of the present invention provides a
15 host cell containing a nucleic acid construct comprising a promoter element, as disclosed herein, operably linked to a heterologous gene. A still further aspect provides a method comprising introducing such a construct into a host cell. The introduction may employ any available technique, including,
20 for eukaryotic cells, calcium phosphate transfection, DEAE-Dextran transfection, electroporation, liposome-mediated transfection and transduction using retrovirus.

The introduction may be followed by causing or allowing expression of the heterologous gene under the control of the
25 promoter, e.g. by culturing host cells under conditions for expression of the gene.

In one embodiment, the construct comprising promoter and gene is integrated into the genome (e.g. chromosome) of the host cell. Integration may be promoted by inclusion in the
30 construct of sequences which promote recombination with the genome, in accordance with standard techniques.

Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are
5 described in detail in *Current Protocols in Molecular Biology*, Second Edition, Ausubel et al. eds., John Wiley & Sons, 1994, the disclosure of which is incorporated herein by reference.

10 Nucleic acid molecules, constructs and vectors according to the present invention may be provided isolated and/or purified (i.e. from their natural environment), in substantially pure or homogeneous form, free or substantially free of a utrophin coding sequence, or free or substantially free of nucleic acid or genes of the species of interest or origin other than the promoter sequence. Nucleic acid according to the present
15 invention may be wholly or partially synthetic. The term "isolate" encompasses all these possibilities.

Nucleic acid constructs comprising a promoter (as disclosed herein) and a heterologous gene (reporter) may be employed in screening for a substance able to modulate utrophin promoter
20 activity. For therapeutic purposes, e.g. for treatment of muscular dystrophy, a substance able to up-regulate expression of the promoter may be sought. A method of screening for ability of a substance to modulate activity of a utrophin promoter may comprise contacting an expression system, such as
25 a host cell, containing a nucleic acid construct as herein disclosed with a test or candidate substance and determining expression of the heterologous gene. The level of transcription of the heterologous gene, or the level of heterologous protein may be determined. The level of protein
30 may be determined by measuring the amount of protein, or the activity of the protein, using techniques known to those skilled in the art.

Alternatively, or additionally a method of screening for

ability of a substance to modulate activity of a utrophin promoter may comprise contacting a cell containing an endogenous utrophin gene (e.g. a mammalian muscle cell) with a test substance and measuring the level of RNA transcription or protein expression using binding members specific for the nucleic acid or polypeptides disclosed herein. Specific binding members include antibodies and nucleic acid probes.

The level of expression in the presence of the test substance may be compared with the level of expression in the absence of the test substance. A difference in expression in the presence of the test substance indicates ability of the substance to modulate gene expression. An increase in expression of the heterologous gene compared with expression of another gene not linked to a promoter as disclosed herein indicates specificity of the substance for modulation of the utrophin promoter.

A promoter construct may be transfected into a cell line using any technique previously described to produce a stable cell line containing the reporter construct integrated into the genome. The cells may be grown and incubated with test compounds for varying times. The cells may be grown in 96 well plates to facilitate the analysis of large numbers of compounds. The cells may then be washed and the reporter gene expression analysed. For some reporters, such as luciferase, the cells will be lysed then analysed. Previous experiments testing the effects of glucocorticoids on the endogenous utrophin protein and RNA levels in myoblasts have already been described [12,13] and techniques used for those experiments may similarly be employed.

Constructs comprising one or more developmental and/or time-specific regulatory motifs (as discussed) may be used to screen for a substance able to modulate the corresponding aspect of the promoter activity, e.g. muscle-specific

expression.

Following identification of a substance which modulates or affects utrophin promoter activity, the substance may be investigated further. Furthermore, it may be manufactured and/or used in preparation, i.e. manufacture or formulation, of a composition such as a medicament, pharmaceutical composition or drug. These may be administered to individuals.

As noted above, the inventors also identified a novel coding sequence (Exon IB) which encodes a novel utrophin N-terminus.

According to a further aspect of the present invention there is provided a nucleic acid molecule which has a nucleotide sequence encoding a polypeptide which includes the amino acid sequence shown in Figure 1 or Figure 2.

Such a polypeptide may include other utrophin sequences, and the nucleic acid molecule may be in the form of a utrophin "mini-gene" (discussed further below).

Such a polypeptide may include non-utrophin (i.e. heterologous or foreign) sequences and thereby form a larger fusion protein. For example, such a fusion protein could be used to target a non-utrophin polypeptide to muscle membranes.

The coding sequence included may be that shown in Figure 1 or Figure 2 or it may be a mutant, variant, derivative or allele of the sequence shown. The sequence may differ from that shown by a change which is one or more of addition, insertion, deletion and substitution of one or more nucleotides of the sequence shown. Changes to a nucleotide sequence may result in an amino acid change at the protein level, or not, as determined by the genetic code.

Thus, nucleic acid according to the present invention may

include a sequence different from the sequences shown in Figure 1 or Figure 2 yet encode a polypeptide with the same amino acid sequence. The amino acid sequences shown in Figure 1 and figure 2 consist of 31 residues.

5 On the other hand the encoded polypeptide may comprise an amino acid sequence which differs by one or more amino acid residues from the amino acid sequences shown in Figure 1 or Figure 2. Nucleic acid encoding a polypeptide which is an amino acid sequence mutant, variant, derivative or allele of
10 the sequences shown in Figure 1 and Figure 2 are further provided by the present invention. Nucleic acid encoding such a polypeptide may show at the nucleotide sequence and/or encoded amino acid level greater than about 60% homology with the coding sequence and/or the amino acid sequence shown in
15 Figure 1 or Figure 2, greater than about 70% homology, greater than about 80% homology, greater than about 90% homology or greater than about 95% homology. Determination of homology is discussed elsewhere herein.

A polypeptide which is a variant, allele, derivative or mutant
20 may have an amino acid sequence which differs from that given in a figure herein by one or more of addition, substitution, deletion and insertion of one or more amino acids. Preferred such polypeptides have wild-type function, that is to say have one or more of the following properties: immunological cross-
25 reactivity with an antibody reactive the polypeptide for which the sequence is given in Figure 1 or Figure 2; sharing an epitope with the polypeptide for which the amino acid sequence is shown in Figure 1 or Figure 2 (as determined for example by immunological cross-reactivity between the two polypeptides);
30 a biological activity which is inhibited by an antibody raised against the polypeptide whose sequence is shown in Figure 1 or Figure 2; ability to bind muscle membrane, ability to bind actin; ability to bind DPC.

Variations in amino acid sequence include "conservative variation", i.e. substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine. Particular amino acid sequence variants may differ from that shown in Figure 1 or Figure 2 by insertion, addition, substitution or deletion of 1 amino acid, 2, 3, 4, or 5-10 amino acids.

According to one aspect of the present invention there is provided a nucleic acid molecule comprising a sequence of nucleotides encoding a polypeptide with utrophin function. Utrophin nucleotide sequences which may be included in the nucleic acid molecule are disclosed in WO 97/922696 which is incorporated herein by reference.

See also Figure 8 and Figure 9 for disclosure of nucleic acid molecules and polypeptides according to the present invention, comprising the exon IB sequence of the invention.

A polypeptide with utrophin function is able to bind actin and able to bind the dystrophin protein complex (DPC).

The nucleic acid molecule may be an isolate, or in an isolated and/or purified form, that is to say not in an environment in which it is found in nature, removed from its natural environment. It may be free from other nucleic acid obtainable from the same species, e.g. encoding another polypeptide.

In one embodiment, nucleic acid molecule is a "mini-gene", i.e. the polypeptide encoded does not correspond to full-length utrophin but is rather shorter, a truncated version (Utrophin mini-genes are discussed in WO97/22696). For instance, part or all of the rod domain may be missing, such

that the polypeptide comprises an actin-binding domain and a DPC-binding domain but is shorter than naturally occurring utrophin. In a full-length utrophin gene including what are identified herein as exons 1A and 1B, the actin-binding domain is encoded by nucleotides 1-739, while the DPC-binding domain (CRCT) is encoded by nucleotides 8499-10301 (where 1 represents the start of translation). See also Figure 8. The respective domains in the polypeptide encoded by a mini-gene according to the invention may comprise amino acids corresponding to those encoded by these nucleotides in the full-length coding sequence. In one embodiment, a minigene according to the present invention comprises or consists of the amino acid sequence encoded by nucleotides 1-739 and 8499-10301 of the A isoform of utrophin in which exon 1B as identified herein is substituted for exons 1A and 2A. The sequence of such a minigene can be constructed by the ordinary skilled person using information disclosed herein, taking into account the content of WO97/22696 and Tinsley et al, *Nature* (1996) 384:349. The nucleic acid sequence and predicted amino acid sequence encoded by a 'mini-gene' according to the present invention are shown in Figure 9.

Advantages of a mini-gene over a sequence encoding a full-length utrophin molecule or derivative thereof include easier manipulation and inclusion in vectors, such as adenoviral and retroviral vectors for delivery and expression.

A further preferred non-naturally occurring nucleic acid molecule encoding a polypeptide with the specified characteristics is a chimaeric construct wherein the encoding sequence comprises a sequence obtainable from one mammal, preferably human ("a human sequence"), and a sequence obtainable from another mammal, preferably mouse ("a mouse sequence"). Such a chimaeric construct may of course comprise the addition, insertion, substitution and/or deletion of one or more nucleotides with respect to the parent mammalian

sequences from which it is derived. Preferably, the part of the coding sequence which encodes the actin-binding domain comprises a sequence of nucleotides obtainable from the mouse, or other non-human mammal, or a sequence of nucleotides
5 derived from a sequence obtainable from the mouse, or other non-human mammal.

In a preferred embodiment, the sequence of nucleotides encoding the polypeptide comprises sequence GAGGCAC at residues 331-337 and/or the sequence GATTGTGGATGAAAACAGTGGG at
10 residues 1453-1475 (using the conventional numbering from the initiation codon ATG), and a sequence obtainable from a human.

Nucleic acid according to the present invention is obtainable using one or more oligonucleotide probes or primers designed to hybridise with one or more fragments of a nucleic acid
15 sequence shown in Figure 1 or Figure 2 particularly fragments of relatively rare sequence, based on codon usage or statistical analysis. The amino acid sequence information provided may be used in design of degenerate probes/primers or "long" probes. A primer designed to hybridise with a fragment
20 of the nucleic acid sequence shown may be used in conjunction with one or more oligonucleotides designed to hybridise to a sequence in a cloning vector within which target nucleic acid has been cloned, or in so-called "RACE" (rapid amplification of cDNA ends) in which cDNA's in a library are ligated to an
25 oligonucleotide linker and PCR is performed using a primer which hybridises with the sequence shown in the figures and a primer which hybridises to the oligonucleotide linker.

Nucleic acid isolated and/or purified from one or more cells (e.g. human, mouse) or a nucleic acid library derived from
30 nucleic acid isolated and/or purified from cells (e.g. a cDNA library derived from mRNA isolated from the cells), may be probed under conditions for selective hybridisation and/or subjected to a specific nucleic acid amplification reaction

such as the polymerase chain reaction (PCR).

A method may include hybridisation of one or more (e.g. two) probes or primers to target nucleic acid. Where the nucleic acid is double-stranded DNA, hybridisation will generally be preceded by denaturation to produce single-stranded DNA. The hybridisation may be as part of a PCR procedure, or as part of a probing procedure not involving PCR. An example procedure would be a combination of PCR and low stringency hybridisation. A screening procedure, chosen from the many available to those skilled in the art, is used to identify successful hybridisation events and isolated hybridised nucleic acid.

Probing may employ the standard Southern blotting technique. For instance DNA may be extracted from cells and digested with different restriction enzymes. Restriction fragments may then be separated by electrophoresis on an agarose gel, before denaturation and transfer to a nitrocellulose filter. Labelled probe may be hybridised to the DNA fragments on the filter and binding determined. DNA for probing may be prepared from RNA preparations from cells.

Preliminary experiments may be performed by hybridising under low stringency conditions various probes to Southern blots of DNA digested with restriction enzymes. Suitable conditions would be achieved when a large number of hybridising fragments were obtained while the background hybridisation was low. Using these conditions nucleic acid libraries, e.g. cDNA libraries representative of expressed sequences, may be searched.

It may be necessary for one or more gene fragments to be ligated to generate a full-length coding sequence. Also, where a full-length encoding nucleic acid molecule has not been obtained, a smaller molecule representing part of the

full molecule, may be used to obtain full-length clones. Inserts may be prepared from partial cDNA clones and used to screen cDNA libraries.

5 Those skilled in the art are well able to employ suitable conditions of the desired stringency for selective hybridisation, taking into account factors such as oligonucleotide length and base composition, temperature and so on. Exemplary conditions have been discussed already above.

10 Nucleic acid according to the present invention may form part of a cloning vector and/or a vector from which the encoded polypeptide may be expressed. Polypeptide expression is discussed below. Suitable vectors can be chosen or constructed, containing appropriate and appropriately
15 positioned regulatory sequences, as discussed elsewhere herein.

A further aspect of the present invention provides a polypeptide which comprises the amino acid sequence shown in Figure 1 or Figure 2. As mentioned earlier such a polypeptide
20 may include other utrophin sequences or may include heterologous sequences.

Polypeptides which are amino acid sequence variants, alleles, derivatives or mutants are also provided by the present invention. Such polypeptides are discussed elsewhere herein.

25 The skilled person can use the techniques described herein and others well known in the art to produce large amounts of peptides, for instance by expression from encoding nucleic acid.

In a further aspect the invention provides a method of making
30 a polypeptide, the method including expression from nucleic

acid encoding the polypeptide (generally nucleic acid according to the invention). This may be conveniently be achieved by growing in culture a host cell containing such a vector, under suitable conditions which cause or allow
5 expression of the polypeptide. Polypeptides may also be expressed in in vitro systems such as reticulocyte lysate.

Systems for cloning and expression of a polypeptide in a variety of different host cells are well known. Suitable host cells include bacteria, mammalian cells, yeast and baculovirus
10 systems. Mammalian cell lines available in the art for expression of a heterologous polypeptide include Chinese hamster ovary cells, HeLa cells, baby hamster kidney cells and many others. A common, preferred bacterial host is *E. coli*.

Thus, a further aspect of the present invention provides a
15 host cell containing heterologous nucleic acid encoding a polypeptide as disclosed herein.

The nucleic acid may be integrated into the genome (e.g. chromosome) of the host cell or may be on an extra-chromosomal vector within the cell, or otherwise identifiably heterologous
20 or foreign to the cell.

A still further aspect provides a method comprising introducing such nucleic acid into a host cell. Suitable techniques are discussed elsewhere herein.

The introduction may be followed by causing or allowing
25 expression from the nucleic acid, e.g. by culturing host cells under conditions for expression of the gene.

The polypeptide encoded by the nucleic acid may be expressed from the nucleic acid *in vitro*, e.g. in a cell-free system or in cultured cells, or *in vivo*.

30 If the polypeptide is expressed coupled to an appropriate signal leader peptide it may be secreted from the cell into

the culture medium.

Peptides can also be generated wholly or partly by chemical synthesis. The compounds of the present invention can be readily prepared according to well-established, standard liquid or, preferably, solid-phase peptide synthesis methods, general descriptions of which are broadly available (see, for example, in J.M. Stewart and J.D. Young, Solid Phase Peptide Synthesis, 2nd edition, Pierce Chemical Company, Rockford, Illinois (1984), in M. Bodanzsky and A. Bodanzsky, The Practice of Peptide Synthesis, Springer Verlag, New York (1984); and Applied Biosystems 430A Users Manual, ABI Inc., Foster City, California), or they may be prepared in solution, by the liquid phase method or by any combination of solid-phase, liquid phase and solution chemistry, e.g. by first completing the respective peptide portion and then, if desired and appropriate, after removal of any protecting groups being present, by introduction of the residue X by reaction of the respective carbonic or sulfonic acid or a reactive derivative thereof.

The present invention also includes active portions, fragments, derivatives and functional mimetics of the polypeptides of the invention. An "active portion" of a polypeptide means a peptide which is less than said full length polypeptide, but which retains a biological activity, such as a biological activity selected from binding to ligand, binding to muscle membrane. Such an active fragment may be included as part of a fusion protein, e.g. including a polypeptide which is to be targetted to the muscle membrane.

A "fragment" of a polypeptide generally means a stretch of amino acid residues of about five to twenty-five contiguous amino acids, typically about ten to twenty contiguous amino acids. Fragments of the novel N-terminus polypeptide sequence may include antigenic determinants or epitopes useful for

raising antibodies to a portion of the amino acid sequence, or may be sequence useful for targetting to muscle membrane. Alanine scans are commonly used to find and refine peptide motifs within polypeptides, this involving the systematic replacement of each residue in turn with the amino acid alanine, followed by an assessment of biological activity.

Preferred fragments of exon 1B polypeptide include those comprising or consisting of an epitope which may be used for instance in raising or isolating antibodies. Variant and derivative peptides, peptides which have an amino acid sequence which differs from one of these sequences by way of addition, insertion, deletion or substitution of one or more amino acids are also provided by the present invention.

A "derivative" of a polypeptide or a fragment thereof may include a polypeptide modified by varying the amino acid sequence of the protein, e.g. by manipulation of the nucleic acid encoding the protein or by altering the protein itself. Such derivatives of the natural amino acid sequence may involve one or more of insertion, addition, deletion or substitution of one or more amino acids, which may be without fundamentally altering the qualitative nature of biological activity of the wild type polypeptide. Also encompassed within the scope of the present invention are functional mimetics of active fragments of the exon 1B polypeptides provided (including alleles, mutants, derivatives and variants). The term "functional mimetic" means a substance which may not contain an active portion of the relevant amino acid sequence, and probably is not a peptide at all, but which retains in qualitative terms biological activity of natural exon 1B polypeptide. The design and screening of candidate mimetics is described in detail below.

A polypeptide according to the present invention may be isolated and/or purified (e.g. using an antibody) for instance

after production by expression from encoding nucleic acid (for which see below). Thus, a polypeptide may be provided free or substantially free from contaminants with which it is naturally associated (if it is a naturally-occurring polypeptide). A polypeptide may be provided free or substantially free of other polypeptides. Polypeptides according to the present invention may be generated wholly or partly by chemical synthesis. The isolated and/or purified polypeptide may be used in formulation of a composition, which may include at least one additional component, for example a pharmaceutical composition including a pharmaceutically acceptable excipient, vehicle or carrier. A composition including a polypeptide according to the invention may be used in prophylactic and/or therapeutic treatment as discussed below.

A polypeptide, peptide, allele, mutant, derivative or variant according to the present invention may be used as an immunogen or otherwise in obtaining specific antibodies. Antibodies are useful in purification and other manipulation of polypeptides and peptides, diagnostic screening and therapeutic contexts.

Accordingly, a further aspect of the present invention provides an antibody able to bind specifically to the polypeptide whose sequence is given in Figure 1 or Figure 2. Such an antibody may be specific in the sense of being able to distinguish between the polypeptide it is able to bind and other human (or mouse) polypeptides for which it has no or substantially no binding affinity (e.g. a binding affinity of about 1000x less). Specific antibodies bind an epitope on the molecule which is either not present or is not accessible on other molecules. Antibodies according to the present invention may be specific for the wild-type polypeptide. Antibodies according to the invention may be specific for a particular mutant, variant, allele or derivative polypeptide as between that molecule and the wild-type polypeptide, so as

to be useful in diagnostic and prognostic methods as discussed below. Antibodies are also useful in purifying the polypeptide or polypeptides to which they bind, e.g. following production by recombinant expression from encoding nucleic acid.

Preferred antibodies according to the invention are isolated, in the sense of being free from contaminants such as antibodies able to bind other polypeptides and/or free of serum components. Monoclonal antibodies are preferred for some purposes, though polyclonal antibodies are within the scope of the present invention.

Antibodies may be obtained using techniques which are standard in the art. Methods of producing antibodies include immunising a mammal (e.g. mouse, rat, rabbit, horse, goat, sheep or monkey) with the protein or a fragment thereof. Antibodies may be obtained from immunised animals using any of a variety of techniques known in the art, and screened, preferably using binding of antibody to antigen of interest. For instance, Western blotting techniques or immunoprecipitation may be used (Armitage et al., 1992, Nature 357: 80-82). Isolation of antibodies and/or antibody-producing cells from an animal may be accompanied by a step of sacrificing the animal.

As an alternative or supplement to immunising a mammal with a peptide, an antibody specific for a protein may be obtained from a recombinantly produced library of expressed immunoglobulin variable domains, e.g. using lambda bacteriophage or filamentous bacteriophage which display functional immunoglobulin binding domains on their surfaces; for instance see WO92/01047. The library may be naive, that is constructed from sequences obtained from an organism which has not been immunised with any of the proteins (or fragments), or may be one constructed using sequences obtained

from an organism which has been exposed to the antigen of interest.

Antibodies according to the present invention may be modified in a number of ways. Indeed the term "antibody" should be construed as covering any binding substance having a binding domain with the required specificity. Thus the invention covers antibody fragments, derivatives, functional equivalents and homologues of antibodies, including synthetic molecules and molecules whose shape mimicks that of an antibody enabling it to bind an antigen or epitope.

Example antibody fragments, capable of binding an antigen or other binding partner are the Fab fragment consisting of the VL, VH, C1 and CH1 domains; the Fd fragment consisting of the VH and CH1 domains; the Fv fragment consisting of the VL and VH domains of a single arm of an antibody; the dAb fragment which consists of a VH domain; isolated CDR regions and F(ab')₂ fragments, a bivalent fragment including two Fab fragments linked by a disulphide bridge at the hinge region. Single chain Fv fragments are also included.

A hybridoma producing a monoclonal antibody according to the present invention may be subject to genetic mutation or other changes. It will further be understood by those skilled in the art that a monoclonal antibody can be subjected to the techniques of recombinant DNA technology to produce other antibodies or chimeric molecules which retain the specificity of the original antibody. Such techniques may involve introducing DNA encoding the immunoglobulin variable region, or the complementarity determining regions (CDRs), of an antibody to the constant regions, or constant regions plus framework regions, of a different immunoglobulin. See, for instance, EP184187A, GB 2188638A or EP-A-0239400. Cloning and expression of chimeric antibodies are described in EP-A-0120694 and EP-A-0125023.

Hybridomas capable of producing antibody with desired binding characteristics are within the scope of the present invention, as are host cells, eukaryotic or prokaryotic, containing nucleic acid encoding antibodies (including antibody fragments) and capable of their expression. The invention also provides methods of production of the antibodies including growing a cell capable of producing the antibody under conditions in which the antibody is produced, and preferably secreted.

10 The reactivities of antibodies on a sample may be determined by any appropriate means. Tagging with individual reporter molecules is one possibility. The reporter molecules may directly or indirectly generate detectable, and preferably measurable, signals. The linkage of reporter molecules may be
15 directly or indirectly, covalently, e.g. via a peptide bond or non-covalently. Linkage via a peptide bond may be as a result of recombinant expression of a gene fusion encoding antibody and reporter molecule.

One favoured mode is by covalent linkage of each antibody with
20 an individual fluorochrome, phosphor or laser dye with spectrally isolated absorption or emission characteristics. Suitable fluorochromes include fluorescein, rhodamine, phycoerythrin and Texas Red. Suitable chromogenic dyes include diaminobenzidine.

25 Other reporters include macromolecular colloidal particles or particulate material such as latex beads that are coloured, magnetic or paramagnetic, and biologically or chemically active agents that can directly or indirectly cause detectable signals to be visually observed, electronically detected or
30 otherwise recorded. These molecules may be enzymes which catalyse reactions that develop or change colours or cause changes in electrical properties, for example. They may be molecularly excitable, such that electronic transitions

between energy states result in characteristic spectral absorptions or emissions. They may include chemical entities used in conjunction with biosensors. Biotin/avidin or biotin/streptavidin and alkaline phosphatase detection systems
5 may be employed.

The mode of determining binding is not a feature of the present invention and those skilled in the art are able to choose a suitable mode according to their preference and general knowledge. Particular embodiments of antibodies
10 according to the present invention include antibodies able to bind and/or which bind specifically, e.g. with an affinity of at least 10^{-7} M, to the peptides shown in Figure 1 or Figure 2.

Antibodies according to the present invention may be used in screening for the presence of a polypeptide, for example in a
15 test sample containing cells or cell lysate as discussed, and may be used in purifying and/or isolating a polypeptide according to the present invention, for instance following production of the polypeptide by expression from encoding nucleic acid therefor.

20 An antibody may be provided in a kit, which may include instructions for use of the antibody, e.g. in determining the presence of a particular substance in a test sample. One or more other reagents may be included, such as labelling molecules, buffer solutions, elutants and so on. Reagents may
25 be provided within containers which protect them from the external environment, such as a sealed vial.

The present invention extends in various aspects not only to a substance identified using a nucleic acid molecule as a modulator of utrophin promoter activity, or to a polypeptide,
30 or nucleic acid molecule in accordance with what is disclosed herein, but also a pharmaceutical composition, medicament, drug or other composition comprising such a substance, a

method comprising administration of such a composition to a patient, e.g. for increasing utrophin expression for instance in treatment of muscular dystrophy, use of such a substance in manufacture of a composition for administration, e.g. for
5 increasing utrophin expression for instance in treatment of muscular dystrophy, and a method of making a pharmaceutical composition comprising admixing such a substance with a pharmaceutically acceptable excipient, vehicle or carrier, and optionally other ingredients.

10 Administration will preferably be in a "therapeutically effective amount", this being sufficient to show benefit to a patient. Such benefit may be at least amelioration of at least one symptom. The actual amount administered, and rate and time-course of administration, will depend on the nature
15 and severity of what is being treated. Prescription of treatment, eg decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors.

A composition may be administered alone or in combination with
20 other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

Pharmaceutical compositions according to the present invention, and for use in accordance with the present invention, may comprise, in addition to active ingredient, a
25 pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on
30 the route of administration, which may be oral, or by injection, e.g. cutaneous, subcutaneous or intravenous.

Pharmaceutical compositions for oral administration may be in

tablet, capsule, powder or liquid form. A tablet may comprise a solid carrier such as gelatin or an adjuvant. Liquid pharmaceutical compositions generally comprise a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included.

For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

Instead of a substance identified using a promoter as disclosed herein, a mimetic or mimick or the substance may be designed for pharmaceutical use. The designing of mimetics to a known pharmaceutically active compound is a known approach to the development of pharmaceuticals based on a "lead" compound. This might be desirable where the active compound is difficult or expensive to synthesise or where it is unsuitable for a particular method of administration, eg peptides are unsuitable active agents for oral compositions as they tend to be quickly degraded by proteases in the alimentary canal. Mimetic design, synthesis and testing may be used to avoid randomly screening large number of molecules for a target property.

There are several steps commonly taken in the design of a mimetic from a compound having a given target property.

Firstly, the particular parts of the compound that are critical and/or important in determining the target property are determined. In the case of a peptide, this can be done by systematically varying the amino acid residues in the peptide, eg by substituting each residue in turn. These parts or residues constituting the active region of the compound are known as its "pharmacophore".

Once the pharmacophore has been found, its structure is modelled to according its physical properties, eg stereochemistry, bonding, size and/or charge, using data from a range of sources, eg spectroscopic techniques, X-ray diffraction data and NMR. Computational analysis, similarity mapping (which models the charge and/or volume of a pharmacophore, rather than the bonding between atoms) and other techniques can be used in this modelling process. In a variant of this approach, the three-dimensional structure of the ligand and its binding partner are modelled. This can be especially useful where the ligand and/or binding partner change conformation on binding, allowing the model to take account of this the design of the mimetic.

A template molecule is then selected onto which chemical groups which mimic the pharmacophore can be grafted. The template molecule and the chemical groups grafted on to it can conveniently be selected so that the mimetic is easy to synthesise, is likely to be pharmacologically acceptable, and does not degrade *in vivo*, while retaining the biological activity of the lead compound. The mimetic or mimetics found by this approach can then be screened to see whether they have the target property, or to what extent they exhibit it. Further optimisation or modification can then be carried out to arrive at one or more final mimetics for *in vivo* or clinical testing.

Mimetics of substances identified as having ability to

modulate utrophin promoter activity using a screening method as disclosed herein are included within the scope of the present invention.

5 Modifications to and further aspects and embodiments of the present invention will be apparent to those skilled in the art. All documents mentioned herein are incorporated by reference.

10 Experimental basis for and embodiments of the present invention will now be described in more detail, by way of example and not limitation, and with reference to the following figures:

Figure 1 shows the sequence of the human exon 1B and promoter B. Numbering corresponds to the insert of pBSX2.0. The deduced translation of exon 1B is shown. The positions of features
15 such as restriction sites, IL-6 response element and Alu repetitive elements are shown.

Figure 2 shows the sequence of the mouse exon 1B and promoter B. Numbering corresponds to the insert of pBSX8.0. The deduced translation of exon 1B is shown. The positions of features
20 such as restriction sites, IL-6 response element and Alu repetitive elements are shown.

Figure 3 shows the sequence alignment of human (top) and mouse (bottom) exon 1B (in upper case) and promoter B. Numbering corresponds to the inserts of pBSX2.0 and pBSX8.0,
25 respectively. The human PvuII site (see Figure 7) is indicated. The open triangle indicates the position at which the luciferase coding sequence was inserted to make pGL3/UtroB/F (see below). The deduced translation of exon 1B is shown; amino acids marked in bold type are identical
30 between the human and mouse sequences. The conserved splice donor consensus is shown in grey. Two putative Apl sites and

an initiator-like element (Inr) are 100% conserved and indicated in black. A solid arrow marks the single transcription start indicated by primer extension; figures adjacent to the sequence indicate the number of individual
5 5'RACE clones that terminated at the positions shown.

Figure 4 shows the position of the primers used in RT-PCR of exon 1B-containing utrophin transcript, and the probes used to probe the PCR products. Primers specific to exon 1B (BF31) and utrophin C-terminus (CT2) were used to amplify 9816bp of
10 utrophin cDNA. The products were blotted and probed with U41, U107, BR4 and U16 as indicated. The diagram is not to scale; numbering refers to the nucleotide sequence of the full-length cDNA. The corresponding functional domains of the protein are indicated above: actin binding domain; rod, rod domain; Cys,
15 cysteine rich domain, C-Term; C-terminal domain.

Figure 5 shows a schematic representation of (A) human YAC and (B) mouse PAC contigs showing position of exons within the genomic map. Key to mouse restriction sites: C, ClaI; S,
20 SacII; B, BssHII; X, XhoI. (C) shows the nomenclature for utrophin promoters, exons and transcripts.

Figure 6 shows the *in vitro* activity of utrophin promoter B. (A) shows normalised luciferase activity following transfection of three different human cell types with either
25 pGL3/utroB/F ('forward construct') or pGL3/utroB/R ('reverse construct').

Figure 7 shows deletion analysis of promoter B. The 1.5kb insert of pGL3/utroB/F was deleted at its 5' and 3' ends using the internal restriction sites indicated. Reporter activity
30 was assayed following transient transfection of IN157 and CL11T47 cells.

Figure 8 shows conceptual translation of exon 1B as part of

utrophin, showing a nucleotide sequence and encoded polypeptide according to embodiments of the present invention.

Figure 9 shows the nucleic acid and predicted amino acid sequence of a utrophin B isoform 'minigene'.

- 5 Figure 10 shows the dosage dependence of IL-6 mediated expression from the isoform B promoter.

Oligonucleotides, PCR, RT-PCR and 5'RACE

PCR and RT-PCR were performed as described (Blake, et al. (1996) *J Biol Chem* 271, 7802-7810). Oligonucleotide sequences (5' to 3') were:

| | | |
|----|------|---------------------------------|
| | UM83 | gatgttcctg tgaggccttc gag, |
| | UM82 | cactcttgga aaatcgagcg t, |
| | U16 | actatgatgt ctgccagagt tg, |
| | U107 | gatccaatag cttccttcca tcttt, |
| 15 | UBF | tggaaaaagt ggagggttgga, |
| | BR2 | tccaacctcc actttttcca, |
| | BR4 | gcctggagag ctacatgccc t, |
| | BF8 | ctccacatct ttttcctcat catct, |
| | BF9 | gattgtggtg atggttgtag aa, |
| 20 | BR10 | gattgtggtg atggttgtag aa, |
| | BR14 | gatgatgagg aaaaagatgt ggag, |
| | BF15 | aaacccaaaa taacacagga catc, |
| | BF16 | agtgtaactt ctctctggtg, |
| | BF31 | taagcagatg taggtgatga gc, |
| 25 | BF42 | gctgcttttg ttgtccactt c, |
| | BR43 | atagcttcct tccatctttg ag, |
| | CT2 | ctccacgttc ttccctctct act, |
| | 2ApF | gcgtgcagtg gaccattttt cagattta, |
| | 1BpF | cgctgcagca gccaccacat ttcgttg, |
| 30 | 3pR | gcgtgcagat cgagcgttta tccatttg. |

5' RACE was undertaken using adapter-ligated mouse heart cDNA (Marathon-Ready, Clontech), following the manufacturer's

protocol, using the supplied adapter primers with nested mouse utrophin primers UM83 (exon 4) and UM82 (exon 3). Products were cloned in pGEM-T (Promega). Human exon 1B was isolated from skeletal muscle cDNA by PCR using mouse primers UBF and UM83. 5'RACE was used to clone the 5' end of human exon 1B, using primers U107 and BR4. Full-length utrophin RT-PCR was done as described (Blake, et al. (1996) *J Biol Chem* 271, 7802-7810.), but using Boehringer Expand Reverse Transcriptase and Long Template PCR reagents, and a primer annealing temperature of 59°C. Semi-quantitative RT-PCR was performed using primers BF42 and BR43 to amplify utrophin B, and commercial primers (Stratagene) to amplify glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Exponential amplification was established by withdrawing samples from thermal cycling at 1 cycle intervals over a range of 5 cycles, predicted to span the exponential range following initial experiments in which samples were withdrawn at 5 cycle intervals. Products were blotted and probed with labelled BR4 or a 600bp GA3PH probe. Band intensities were quantified using a Storm phosphoimager. A graph of \log_2 [band intensity] versus cycle number showed a linear relationship with gradient ≈ 1 , indicating near-perfect exponential amplification. The band intensities at any given cycle over this range are therefore directly proportional to the amount of cDNA in the original samples.

25 Genomic Mapping and Clones

Human YACs are as previously described (Pearce, et al. (1993) *Hum Mol Genet* 2, 1765-72). Southern blots of restriction digested YAC DNA were probed with end-labelled BR4. A 3.0kb hybridising XbaI fragment was cloned from YAC 4X124H10 (a YAC clone which contains a human genomic DNA insert) into pBlueScript (Stratagene) generating pBSX2.0. Mouse PACs were identified from the RPCI21 library. A 398bp exon 1B/promoter B DNA probe (UB400) encompassing human positions 1129 to 1527 was used for exon 1B mapping. Library filters were screened with probes to exons 1A-5 (Dennis, et al. (1996) *Nucleic Acid*

Res 24, 1646-52) and UB400. Eleven PACs were identified, and four of these arranged into a contig by restriction mapping. An 8.0kb XbaI fragment from PAC 110C24, that hybridised with UB400, was cloned in pBlueScript generating pBSX8.0.

5 Northern Blots and Probes

A human multiple tissue northern blot and b-actin control cDNA probe were obtained from Clontech. A utrophin C-terminal cDNA probe, encompassing the last 4.0kb of the utrophin message, was generated by PCR. Human exon 1B sequence between positions
10 1480 and 1596 was cloned into pGEM-T and an exon 1B antisense riboprobe was transcribed (In Vitro Transcription Kit, Promega) from the SP6 promoter following linearisation of the plasmid with NcoI. Hybridisation was carried out at 70°C in 50% formamide hybridisation buffer (Ausubel, et al. (1999)
15 *Current Protocols in Molecular Biology* (Wiley).) and the filter was washed at 75°C in 0.1xSSC, 0.1%SDS for 2 hours.

RNase Protection

Specific probes spanning the exon 1B/3 and exon 2A/3 boundaries were obtained by PCR amplification of mouse heart
20 cDNA using primers 2ApF, 1BpF and 3pR. Products were cloned in the PstI site of pDP18 (Ambion) and sequenced. Plasmids were linearised with EcoRI (1B) or BamHI (2A); labelled antisense riboprobe was transcribed from the T7 promoter and gel purified. RNase protection was carried out using RPAIII
25 kit (Ambion) following the manufacturer's instructions (30µg total RNA unless stated, hybridisation temperature 42°C, RNase A/T1 dilution 1:200). Following electrophoretic separation, band intensities were quantified as above, and corrected for the amount of label present in each protected fragment.

30 Promoter/Reporter Constructs

Reporter constructs were generated by PCR amplification of the human sequence between positions 39 and 1503, using pBSX2.0 as template. Pfu polymerase was used with primers BF9 and BR14.

Following 15 cycles of 96°C for 45 seconds, 62°C for 45 seconds, 72°C for 4 minutes, products were dA-tailed and cloned in pGEM-T. Clones were identified with product in both orientations and insert, liberated by digestion with SacI/NcoI, was cloned into the SacI/NcoI sites of a promoterless luciferase reporter plasmid (pGL3 basic, Promega), generating constructs with insert in forward (pGL3/utroB/F) and reverse (pGL3/UtroB/R) orientation with respect to the coding sequence of luciferase. Deletions of the forward construct were generated by cleavage at SpeI, NdeI, EcoRI and PvuII sites in the insert, followed by religation to sites in the 5' or 3' polylinker. Constructs were sequenced completely.

Cell Culture and Transfections

Three human cell lines (IN157 rhabdomyosarcoma (Nielsen et al., 1993, *Mol Cell Endocrinol* 93: 87-95), CL11T47 kidney epithelial and HeLa cervical epithelial (*Cancer Research*, 1952 12: 264) were maintained as described (Dennis, et al. (1996) *Nucleic Acid Res* 24, 1646-52). 2µg pGL3/utroB/F or R, or its molar equivalent, mixed with 0.5µg of LacZ control plasmid (pSV-β-gal, Promega) was transfected in each well of 6 well plates using Superfect (Qiagen), following the manufacturer's protocol. 48 hours later, cells were harvested and cell extracts were assayed for luciferase and β-galactosidase activity as described (Dennis, et al. (1996) *Nucleic Acids Res* 24, 1646-52). Luciferase activity was standardised to β-galactosidase activity in each individual sample to control for transfection efficiency. Results are expressed as mean luciferase/β-galactosidase ratio for four individual transfections. Error bars indicate the standard error of the mean. For comparison of different constructs within the same cell line, results were standardised to those obtained with pGL3/utroB/F and are expressed as % of this value. For comparison of constructs between cell lines, results were standardised to those obtained with a luciferase-SV40

promoter/enhancer plasmid (pGL3 control, Promega) that generates high levels of reporter activity in all cell lines tested.

Primer Extension

5 Primer extension was carried out as described (18); end-labelled primer BR2 was annealed to 0, 30 or 50 μ g mouse heart total RNA at 58°C for 20 minutes, and extended at 42°C for 40 minutes. Products were separated on a 6% polyacrylamide gel, under denaturing conditions, alongside a sequencing ladder
10 generated from pBSX8.0 using primer BR2.

Results

An alternative 5' exon in utrophin mRNA

Utrophin from a mouse heart cDNA library was amplified by 5'RACE, and the resulting products cloned and sequenced. Of 12
15 clones, 8 contained novel sequence 5' of exon 3. Below, we present evidence that the novel sequence is a single alternative 5' exon of utrophin containing a translational initiation codon. We refer to this sequence as 'exon 1B' to distinguish it from the previously described 5' cDNA sequence
20 comprising untranslated exon 1A and exon 2A which contains the translational start (Figure 5c).

Figure 3 shows a sequence comparison of human and mouse exon 1B, and genomic flanking sequence. The position and phase of the splice junction at the 5' end of exon 3 is identical for
25 both exon 1B- and exon 2A- containing transcripts. Exon 1B contains a putative ATG translation initiation codon and open reading frame, in-frame with that of exon 3, predicting a novel 31 amino acid N-terminus to the utrophin protein. The context of the ATG codon is predicted to be favourable for
30 translation in that there is a purine at position -3 (bold in Figure. 3) (33). Human and mouse exons 1B show 82% nucleotide identity. The predicted translations are 84% identical and 94%

similar. The position and context of the ATG codon are conserved. The human sequence contains a second putative ATG codon immediately 5' (position 1511, solid bar in Figure.1), followed by a TAG stop codon. As this ATG does not adhere to the Kozak consensus, is not associated with an open reading frame and is not present in the mouse sequence, we predict that this is not a functional translation start. A similar feature is present in human exon 2A, where the 5'UTR contains a short open reading frame prior to the true translation start.

The transcript associated with exon 1B

A human multiple tissue northern blot was probed with an exon 1B anti-sense riboprobe. A single hybridising 13kb band was observed, identical to that produced by probing the same blot with a cDNA encompassing 4kb of the utrophin C-terminus, indicating that exon1B is exclusively associated with a full-length utrophin mRNA. Exon 1B is ubiquitously expressed, and appears most abundant in heart and pancreas, and least abundant in the brain, relative to β -actin. This is similar to the expression profile of total full-length utrophin.

RT-PCR was employed to confirm the association of exon 1B with a utrophin mRNA predicted to give rise to functional protein (Figure.4). Amplification of first strand cDNA from IN157 cells utilising a forward primer specific to exon 1B (BF3I) and a reverse primer within the utrophin C-terminus (CT2) produced a product of expected size. Successive hybridisation of this PCR product with domain-specific probes; U41, UBR4, U107 and U16, confirmed that exon 1B is associated with a utrophin transcript spanning the full coding sequence of the gene.

The expression profiles of exons 1B and 2A were examined using RNase protection. Specific riboprobes corresponding to the exon 1B/3 and 2A/3 boundaries were simultaneously hybridised with total RNA, allowing direct quantitation of transcript

abundance. B-utrophin is the most abundant form in the heart, whereas exon 2A-containing transcripts predominate in the kidney. Approximately equal amounts of exons 1B and 2A were observed in the brain and in skeletal muscle.

5 **Mapping and cloning of genomic sequence associated with exon 1B**

Using probe BR4, exon 1B was mapped within our previously described human YAC contig (26) encompassing the 5' end of the utrophin locus (Figure.5a). A hybridising band was seen with
10 YAC 4X124H10 but not 4X23E3 or 5C2 indicating that exon 1B lies within the 120kb intron 2 of the utrophin gene. A subsequent database search identified a clone from the HGMP human chromosome 6 sequencing project, containing exons 1A, 2A and 1B. This indicated that exon 1B lies 52.2kb 3' of exon 2A
15 (Figure.5a). Probing the mouse genomic PAC library (RPCI21 from P. DeJong, Roswell Park Cancer Institute) with utrophin exons 1A, 1B and 2- 5 inclusive identified a series of genomic PACs spanning the 5' end of the mouse utrophin gene. Four of these PACs were assembled into a contig of the region.
20 Hybridisation with UB400 confirmed that exon 1B lies within intron 2 in the mouse (Figure.5b), approximately 50kb 3' of exon 2.

Human and mouse genomic fragments were obtained from the YAC and PAC libraries, respectively. Genomic sequence
25 encompassing exon 1B was obtained by an Xba I digest of YAC 4X124H10 (human 3kb fragment) and PAC110c24 (mouse 8.8kb fragment). These fragments were sub-cloned into pBluescript vector, the human fragment was deleted to 2kb during the sub-cloning. The plasmid clones were designated pBSX2.0 (human)
30 and pBSX8.0 (mouse). Comparison of the cDNA and genomic sequence showed no evidence of a further 5' exon in the transcript associated with exon 1B, suggesting that the genomic flanking sequence contained the transcription start and promoter element responsible for exon 1B expression. Our

nomenclature for utrophin 5' exons, transcripts and promoters appears in Figure 5c.

Promoter B

1.5kb of human genomic sequence 5' of exon 1B, including the 5'UTR of exon 1B, was cloned in both orientations into a promoterless luciferase reporter vector. Three human cell lines (IN157 rhabdomyosarcoma, CL11T47 kidney epithelial and HeLa cervical epithelial) were transiently transfected with these constructs. These three lines were chosen because they are known to express utrophin mRNA and protein at different levels. Reporter activity was detected at significantly higher levels in cells transfected with the forward than the reverse orientation construct, indicating promoter activity (Figure 6). Interestingly, the level of activity varied between cell lines by an order of magnitude. Semi-quantitative RT-PCR demonstrated that the variation of luciferase expression mimicked the transcription profile of endogenous utrophin exon 1B. In contrast, the GA3PDH control showed identical amplification in all cDNA samples, indicating that the differences seen in B-utrophin amplification have arisen from differences in the level of expression of the endogenous B-utrophin transcript in these cell lines. These data show that the 1.5kb of genomic sequence 5' of exon 1B utilised in these reporter clones contains the necessary signals to initiate transcription of exon 1B, and regulatory elements that determine the level of expression in these cell lines.

To further delineate important elements within this region, a series of 5' and 3' deletions of promoter B were made, and the *in vitro* activity of each one assayed (Figure 7). A 300bp element, contained within clone pGL3/utroB/F/D5' Pvu 1199, retains 70% activity of the full 1.5kb construct in expressing cell lines, and shows 74% identity between human and mouse (Figure.3). Homology falls to 50% when sequence further 5' if the human PvuII site is compared with corresponding mouse

sequence using a 35bp window. Homology was determined using GAP, from version 20 of GCG, with default parameters as noted already above.

Promoter B transcription start site

5 The 5' ends of 8 human and 4 mouse 5'RACE clones clustered around a putative cap site in the genomic sequence (Figure.3). None of the 5'RACE clones generated by amplification across the exon 3/exon 1B boundary extended further upstream. RT-PCR was carried out using forward primers around this region with
10 a reverse primer in exon 4. A product of expected size was amplified from IN157 cDNA by primers BF42 and BF8, but not BF16 or BF15, indicating that the transcription start is within the 18bp that separates the two primers BF15 and BF42. These 18 bases contain the putative cap site and the cluster
15 of RACE clone 5' ends.

To map the start site accurately, primer extension using an exon 1B reverse primer and mouse heart RNA was employed. This yielded a single product, indicative of a single transcription start site. Transcription initiates at mouse position 1183
20 within a 25-bp motif, which is 100% conserved between human and mouse. Part of this motif, spanning the cap site, is a 6/7 base match for the initiator consensus, and correspondingly shows homology to the initiators of other genes. The transcription start site is homologous to the initiators of
25 other promoters. Consensus 1, initiator consensus derived from sequence comparison of Inr⁺ genes (Azizkhan, et al. (1993) *Critical Reviews in Eukaryotic Gene Expression* 3, 229-254.); consensus 2, experimentally-derived consensus for functional initiator (Javahery, et al. (1994) *Molecular and Cellular*
30 *Biology* 14, 116-127.); TdT, terminal deoxynucleotidyl transferase; hRAR, human retinoic acid receptor α ; mCREB, mouse cAMP response element binding protein. Transcribed sequence is indicated in bold uppercase. We consider this promoter to be of the TATA-Inr⁺ type.

Assaying for substances which modulate utrophin promoter activity

Method 1:

This method uses a mouse *mdx*-H2K myoblast line stably
5 transfected with a human 7.0kb utrophin promoter-luciferase
construct. On day 1 myoblast cells transfected with the
construct are plated out in 6-well dishes, with compound or
DMSO-only for the negative controls.

4 x 6 well plates are used for every 3 compounds (the
10 compounds are dissolved in DMSO and stored prior to use). For
example, compound A, or B, or C were each added to 1 well,
while the remaining 3 wells contain only DMSO. This results
in 4 wells containing each compound and 12 wells with DMSO
alone. Due to the inherent noise of both the harvesting/assay
15 and cell seeding/growth steps, this is the minimum number that
results in meaningful analysis. Setting up the plates in this
way means that the data really are paired, and can be analysed
with a paired student T test. This provides a more powerful
statistical analysis rather than putting each compound on a
20 different plate and comparing it with a control plate.

On Day 4 the cells are harvested and luciferase quantitation
and pairwise analysis is carried out.

Method 2:

Compounds which up-regulate the endogenous utrophin promoter
25 are be found using *mdx*-H2K myoblasts that are not transfected
with the utrophin promoter-luciferase construct. *Mdx*-
myoblasts can be used to mimic utrophin transcritption and
protein stability in dystrophin-deficient cells.

Identification of utrophin protein expression

30 Quantitative Western Blotting is used to measure the level of

utrophin expression (Tinsley JM, et al., *Nature Medicine* 4, 1441-1444.) Using 6 well plates and treating with compound as described above generates enough total protein sample to test by Western blotting. Antibodies specific to the A protein or
5 B protein are used to quantify levels of either protein.

Identification of utrophin RNA expression

Quantitative ribonuclease protection is used to analyse levels of utrophin expression. A pairwise design is used, as described above, but more cells are necessary. To see bands
10 clearly, about 20-30 μ g total RNA is used. Each compound and control will need a 175 cm² tissue culture flask. A dual probe to simultaneously identify the A transcript and B transcript is be used.

Using the two techniques described compounds are identified
15 after cell treatment which modulate utrophin levels. The same techniques are used for *in vivo* animal experiments where the compound is administered to dystrophin deficient *mdx* mice.

Interleukin-6 (IL-6) Interactions

Two related elements are present in the promoters of genes
20 encoding acute phase proteins that mediate an increase in transcription stimulated by an IL-6 triggered signalling cascade (Hocke et al., 1992). One of these was found to be present in the exon 1B flanking sequence. Wild type and mutated reporter fusions for IL-6 were therefore tested for
25 responsiveness in appropriate cell systems.

Constructs of the 1.5F B promoter normal and mutant (consensus change : ctggaa > gatatc^a concerning the mutant: Hattori M et al (1990) *Proc. Natl. Acad. Sci. U S A.* Mar;87(6):2364-8.) were introduced into a promoter-less luciferase reporter vector and
30 transfected into IN157 cells with a renilla firefly control. Cells were washed and charcoal stripped serum added 5 hours

post-transfection and left overnight. IL-6 amounts were added as illustrated with an appropriate amount of IL-6 soluble receptor. The cells were left for 24 hours and then assayed for activity using a luminometer.

- 5 A dosage dependent transcriptional response was noted in the normal, but not the mutated reporter construct (figure 10). This result indicates the existence of a cytokine mediated signalling pathway which causes up-regulation of the B utrophin promoter through the interaction of IL-6 and IL-6 receptor with
10 the conserved IL-6 response element.

Discussion

- We have demonstrated that there is a second promoter within intron 2 of the utrophin gene, driving expression of a unique first exon that splices into a common 13kb mRNA. These data are
15 important, both in terms of understanding the molecular physiology of utrophin expression, and in view of their application to therapeutic intervention in DMD.

- The functional consequences of genes having more than one promoter have been postulated (reviewed in (Ayoubi, et al
20 (1996) *FASEB J.* 10,453-460). A single gene may achieve a complex temporal and spatial expression pattern by interaction of different promoters with discrete subsets of transcription factors. Dystrophin is an example: three dissimilar promoters are active at different levels in specific cell types within
25 the heart, skeletal muscle and the brain (Gorecki, et al. (1992) *Hum Mol Genet* 1, 505-510., Barnea, et al. (1990) *Neuron* 5, 881-888, Holder, et al. *Human Genetics* 97, 232-239). Northern blot analysis, however, indicates that utrophin exon 1B is ubiquitously expressed, implying that promoters A and B
30 are co-expressed in many tissues. It is conceivable that examination of transcript distribution in whole tissue samples has masked cell type-specific patterns of expression. Data

from isolated human cell lines *in vitro* support this notion; we observed large differences in promoter B activity between different cell lines, consistent with an *in vivo* expression profile involving specific cellular populations.

5 Alternatively, the two promoters may be spatially regulated at a sub-cellular level. Within adult skeletal muscle fibres, promoter A is synaptically driven (Gramolini, *et al.* (1997) *J Biol Chem* 272, 8117-20.), yet aggregates of utrophin mRNA are detectable at up to 25% extrasynaptic nuclei (Vater, *et al.*
10 (1998) *Molecular and cellular Neuroscience* 10, 229-242). Expression of promoter B in the extrasynaptic compartment might be invoked as one possible explanation.

A second proposed function of alternative promoters is the generation of transcripts with interchangeable 5' exons,
15 giving rise to mRNAs with alternative 5'UTRs or proteins with novel N-terminal domains. Unlike exon 1B, utrophin exon 1A contains a long GC-rich 5'UTR. In some transcripts, GC-rich 5'UTRs are not translated efficiently (Kozak, M. (1991) *J Cell Biol* 115, 887-903.), and there are examples of genes in which
20 alternative use of GC-rich and non-GC-rich 5'UTRs has been implicated in post-transcriptional regulation of protein synthesis (Nielson, *et al.* (1990) *J Biol Chem* 265, 13431-13434.). In addition, the predicted 31 amino acids encoded by exon 1B are different to the 26 amino acids of exon 2A; the
25 functions of the resulting N-termini may be different.

The discovery of a second promoter provides a new target for the upregulation of utrophin to ameliorate the DMD phenotype. Promoter B is highly regulated, probably by different factors from promoter A, including IL-6. Elucidation of the mechanisms
30 responsible for the large difference in promoter B activity between IN157 and HeLa cells might lead to identification of a factor that can be delivered to muscle to activate utrophin expression. Importantly, as the N-box motif is absent from promoter B, this is unlikely to carry any risk of NMJ

disruption potentially inherent in the pharmacological manipulation of synaptically regulated promoter A.

CLAIMS

1. An isolated nucleic acid comprising a promoter which comprises a sequence of nucleotides selected from (i) the human promoter sequence shown in Figure 1 and (ii) the mouse promoter sequence shown in Figure 2, free or substantially free of utrophin coding sequence.
2. An isolated nucleic acid consisting essentially of a promoter which comprises the sequence of nucleotides shown 5' to position 1440 in Figure 1.
3. An isolated nucleic acid consisting essentially of a promoter which comprises the sequence of nucleotides shown 5' to position 1183 of the mouse sequence shown in Figure 2.
4. An isolated nucleic acid consisting essentially of a promoter which comprises the nucleotides numbered 1199 -1440 in the sequence shown in Figure 1.
5. An isolated nucleic acid consisting essentially of a promoter which comprises the nucleotides numbered 959-1183 in the sequence shown in Figure 2.
6. An isolated nucleic acid consisting essentially of a promoter which comprises the nucleotide sequence
ACAGGACATCCCAGTGTGCAGTTCG.
7. An isolated nucleic acid consisting essentially of a promoter which comprises a sequence of nucleotides that is an allele, mutant or derivative, by way of addition, insertion, deletion or substitution of one or more nucleotides, of the promoter sequence shown in Figure 1, which sequence has at least 60% homology with the promoter sequence shown in figure 1 and which promoter, when operably linked to a sequence of nucleotides, has the ability to initiate transcription of that

sequence, said transcription being muscle-specific.

8. An isolated nucleic acid consisting essentially of a promoter which comprises a sequence of nucleotides that is an allele, mutant or derivative, by way of addition, insertion, deletion or substitution of one or more nucleotides, of the promoter sequence shown in Figure 2, which sequence has at least 60% homology with the promoter sequence shown in figure 2 and which promoter, when operably linked to a sequence of nucleotides, has the ability to initiate transcription of that sequence, said transcription being muscle-specific.

9. An isolated nucleic acid consisting essentially of a promoter which comprises a sequence of nucleotides that is an allele, mutant or derivative, by way of addition, insertion, deletion or substitution of one or more nucleotides, of the promoter sequence shown in Figure 2, which hybridises to the promoter sequence shown in figure 2 under stringent hybridisation conditions and which promoter, when operably linked to a sequence of nucleotides, has the ability to initiate transcription of that sequence, said transcription being muscle-specific.

10. A nucleic acid construct comprising an isolated nucleic acid according to any of the preceding claims operably linked to a heterologous sequence.

11. A nucleic acid construct according to claim 10 wherein the heterologous sequence is a coding sequence.

12. A nucleic acid construct according to claim 11 wherein the heterologous sequence encodes a reporter molecule.

13. A host cell comprising a nucleic acid construct according to any of claims 10 to 12.

14. A method comprising culturing a host cell according to claim 13 under conditions for transcription of said heterologous sequence from the promoter.

5 15. A method according to claim 14 wherein the heterologous sequence is a coding sequence and the host cell is cultured under conditions for expression of the encoded peptide or polypeptide product.

16. A method according to claim 14 or claim 15 comprising detection of transcription of the heterologous sequence.

10 17. A method according to claim 14 or claim 15 comprising detection of expression of the encoded peptide or polypeptide product.

15 18. A method of screening for a substance able to modulate utrophin promoter activity, the method comprising contacting an expression system containing a nucleic acid construct according to any of claims 10 to 12 with a test or candidate substance and determining transcription of the heterologous sequence or expression of the encoded peptide or polypeptide product.

20 19. A method according to claim 18 wherein the expression system comprises a host cell containing said nucleic acid construct.

25 20. A method which comprises, following identification of a substance able to modulate utrophin promoter activity in accordance with a method according to claim 18 or claim 19, manufacture of the substance and/or use of the substance in manufacture or formulation of a composition.

30 21. The use of an isolated nucleic acid according to any of claims 1 to 6 for promoting transcription of an operably

linked sequence of nucleotides.

22. The use of claim 21 wherein the transcription is tissue-specific, with the tissue-specificity being muscle-specific.

5 23. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide including the amino acid sequence shown in Figure 1 or Figure 2.

10 24. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide that is an allele, mutant or derivative of a polypeptide including the amino acid sequence shown in Figure 1, which amino acid sequence has at least 60% homology with the polypeptide sequence in Figure 1 or Figure 2.

15 25. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide that is an allele, mutant or derivative of a polypeptide shown in Figure 1 or Figure 2, which nucleotide sequence hybridises with the nucleotide sequence encoding the polypeptide in Figure 1 or Figure 2 under stringent hybridisation conditions.

20 26. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide having the amino acid sequence shown in Figure 9.

25 27. An isolated nucleic acid molecule comprising the nucleotide sequence shown in figure 9.

28. Nucleic acid of any one of claims 23 to 27 comprised in a vector.

29. Nucleic acid according to claim 28 wherein said vector is an expression vector.

30. A host cell containing heterologous nucleic acid according to any one of claims 23 to 29.

31. A cell according to claim 30 which is a muscle cell.

5 32. A cell according to claim 30 wherein said polypeptide is expressed.

33. A cell according to any of claims 30 to 32 which is in a mammal.

34. A non-human mammal having a cell according to any of claims 30 to 32.

10 35. A non-human mammal containing nucleic acid according to any of claims 23 to 29.

36. A method including introduction of nucleic acid according to any of claims 23 to 29 into a cell.

15 37. A method according to claim 36 wherein said introduction takes place *in vitro*.

38. A method which includes causing or allowing expression of the coding nucleotide sequence of heterologous nucleic acid according to any of claims 23 to 29 in a cell.

20 39. A method according to claim 38 wherein the cell is part of a mammal.

40. A method according to claim 38 wherein the expression product is purified and/or isolated following expression.

25 41. A method according to claim 40 wherein the expression product is formulated into a composition which includes at least one additional component, following purification and/or

isolation of the expression product.

42. An isolated polypeptide as encoded by nucleic acid according to any of claims 23 to 29.

43. An isolated utrophin exon IB polypeptide selected from:

- 5 (i) human utrophin exon IB polypeptide of which the amino acid sequence is shown in Figure 1;
(ii) mouse utrophin exon IB of which the amino acid sequence is shown in Figure 1.

10 44. An isolated polypeptide including the human polypeptide according to claim 43.

45. An isolated polypeptide including the mouse polypeptide according to claim 44.

15 46. An isolated polypeptide which has 60 % homology with the polypeptide according to claim 44 or 45.

47. An isolated fragment of a polypeptide according to claim 43, which fragments is 5 to 25 amino acids in length.

48. An isolated fragment of a polypeptide according to claim 43, which fragment is 10 to 20 amino acids in length.

20 49. An antibody specific for a polypeptide according to any one of claims 42 to 48.

50. A composition including a polypeptide according to any one of claims 42 to 46, a fragment according to claim 47 or claim 48, or an antibody according to claim 49, and a
25 pharmaceutically acceptable excipient.

51. Use of nucleic acid according to any of claims 23 to 29 in the manufacture of a medicament for treating a dystrophin

phenotype in a mammal.

52. Use of a polypeptide according to any of claims 42 to 48 or an antibody according to claim 49 in the manufacture of a medicament for treating a dystrophin phenotype in a mammal.

11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279 280 281 282 283 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298 299 300 301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500 501 502 503 504 505 506 507 508 509 510 511 512 513 514 515 516 517 518 519 520 521 522 523 524 525 526 527 528 529 530 531 532 533 534 535 536 537 538 539 540 541 542 543 544 545 546 547 548 549 550 551 552 553 554 555 556 557 558 559 560 561 562 563 564 565 566 567 568 569 570 571 572 573 574 575 576 577 578 579 580 581 582 583 584 585 586 587 588 589 590 591 592 593 594 595 596 597 598 599 600 601 602 603 604 605 606 607 608 609 610 611 612 613 614 615 616 617 618 619 620 621 622 623 624 625 626 627 628 629 630 631 632 633 634 635 636 637 638 639 640 641 642 643 644 645 646 647 648 649 650 651 652 653 654 655 656 657 658 659 660 661 662 663 664 665 666 667 668 669 670 671 672 673 674 675 676 677 678 679 680 681 682 683 684 685 686 687 688 689 690 691 692 693 694 695 696 697 698 699 700 701 702 703 704 705 706 707 708 709 710 711 712 713 714 715 716 717 718 719 720 721 722 723 724 725 726 727 728 729 730 731 732 733 734 735 736 737 738 739 740 741 742 743 744 745 746 747 748 749 750 751 752 753 754 755 756 757 758 759 760 761 762 763 764 765 766 767 768 769 770 771 772 773 774 775 776 777 778 779 780 781 782 783 784 785 786 787 788 789 790 791 792 793 794 795 796 797 798 799 800 801 802 803 804 805 806 807 808 809 810 811 812 813 814 815 816 817 818 819 820 821 822 823 824 825 826 827 828 829 830 831 832 833 834 835 836 837 838 839 840 841 842 843 844 845 846 847 848 849 850 851 852 853 854 855 856 857 858 859 860 861 862 863 864 865 866 867 868 869 870 871 872 873 874 875 876 877 878 879 880 881 882 883 884 885 886 887 888 889 890 891 892 893 894 895 896 897 898 899 900 901 902 903 904 905 906 907 908 909 910 911 912 913 914 915 916 917 918 919 920 921 922 923 924 925 926 927 928 929 930 931 932 933 934 935 936 937 938 939 940 941 942 943 944 945 946 947 948 949 950 951 952 953 954 955 956 957 958 959 960 961 962 963 964 965 966 967 968 969 970 971 972 973 974 975 976 977 978 979 980 981 982 983 984 985 986 987 988 989 990 991 992 993 994 995 996 997 998 999 1000 1001 1002 1003 1004 1005 1006 1007 1008 1009 1010 1011 1012 1013 1014 1015 1016 1017 1018 1019 1020 1021 1022 1023 1024 1025 1026 1027 1028 1029 1030 1031 1032 1033 1034 1035 1036 1037 1038 1039 1040 1041 1042 1043 1044 10



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- (81) **Designated States (national):** AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) **Designated States (regional):** ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- Published:**
- *With international search report.*
 - *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

[Continued on next page]

(54) Title: UTROPHIN GENE PROMOTER

[illegible]

(S7) Abstract: Second promoter for mouse and human utrophin genes. The promoters or fragments and derivatives may be used to control transcription of heterologous sequences, including coding sequences of reporter genes. Expression systems such as host cells containing nucleic acid constructs which comprise a promoter as provided operably linked to a heterologous sequence may be used to screen substances for ability to modulate activity of the utrophin promoter. Substances with such ability may be manufactured and/or used in the preparation of compositions such as medicaments. Up-regulation of utrophin expression may compensate for dystrophin loss in muscular dystrophy patients.

WO 01/25461 A1

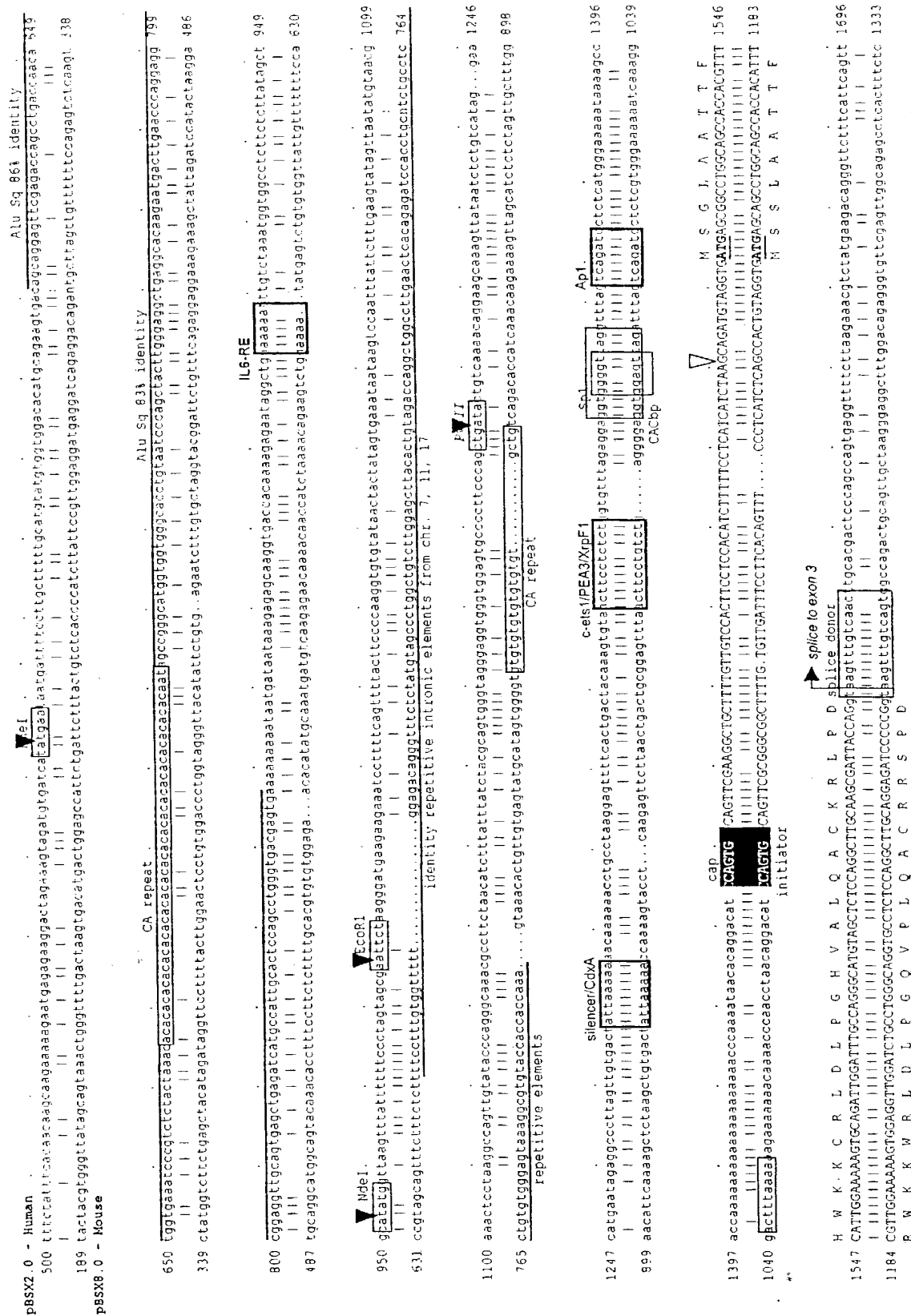


Figure 3

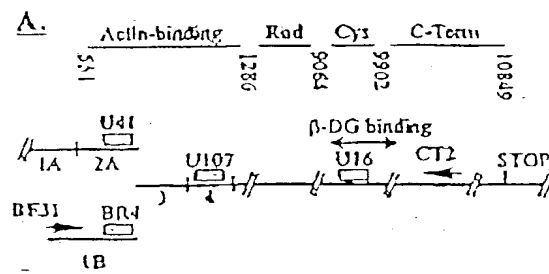


Figure 4

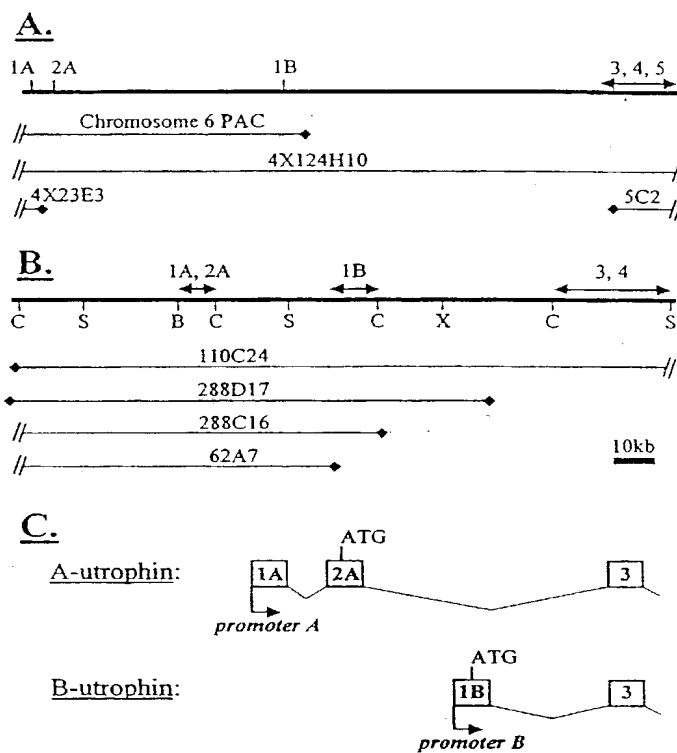


Figure 5

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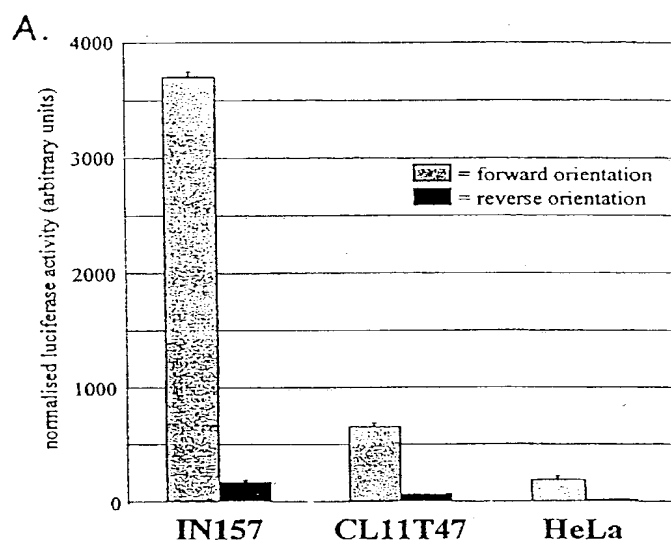


Figure 6

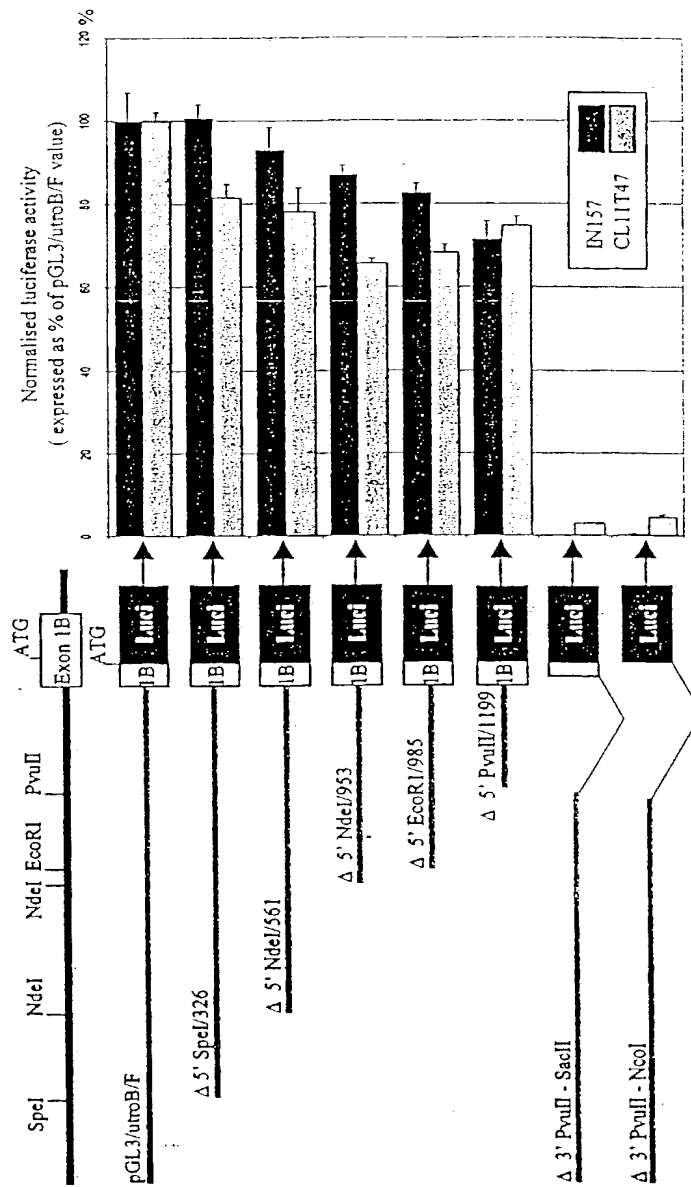


Figure 7

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Human B-utrophin up to nucleotide 1500, deduced translation

```

CCCAGTGTGCAGTTCGAAGGCTGCTTTTGTGTGCTCACTTCCCTCCACATCTTTTTCCTCAT
1  -----+-----+-----+-----+-----+-----+-----+-----+ 60
GGGTCACACGTCAAGCTTCCGACGAAAACACAGGTGAAGGAGGTGTAGAAAAAGGAGTA

CATCTAAGCAGATGTAGGTGATGAGCGGCCTGGCAGCCACCACGTTTCATTGGAAAAAGT
61  -----+-----+-----+-----+-----+-----+-----+-----+ 120
GTAGATTCTGTCTACATCCACTACTCGCCGGACCGTCGGTGGTGCAAAGTAACCTTTTCA

M S G L A A T T F H W K K C -

GCAGATTGGATTGGCCAGGGCATGTAGCTCTCCAGGCTTGCAAGCGATTACCAGATGAAC
121 -----+-----+-----+-----+-----+-----+-----+-----+ 180
CGTCTAACCTAAACGGTCCCGTACATCGAGAGGTCCGAACGTTGCTAATGGTCTACTTG

C      R L D L P G H V A L Q A C K R L P D E H -

ACAATGACGTACAGAAGAAAACCTTTACCAAATGGATAAATGCTCGATTTTCAAAGAGTG
181 -----+-----+-----+-----+-----+-----+-----+-----+ 240
TGTTACTGCATGTCTTCTTTTGGAAATGGTTACCTATTTACGAGCTAAAAGTTTCTCAC

C      N D V Q K K T F T K W I N A R F S K S G -

GGAAACCACCCATCAATGATATGTTACAGACCTCAAAGATGGAAGGAAGCTATTGGATC
241 -----+-----+-----+-----+-----+-----+-----+-----+ 300
CCTTTGGTGGGTAGTTACTATACAAGTGCTGGAGTTTCTACCTTCCTTCGATAACCTAG

C      K P P I N D M F T D L K D G R K L L D L -

TTCTAGAAGGCTCACAGGAACATCACTGCCAAAGGAACGTGGTTCCACAAGGGTACATG
301 -----+-----+-----+-----+-----+-----+-----+-----+ 360
AAGATCTTCCGGAGTGTCTTGTAGTGACGGTTTCTTGCACCAAGGTGTTCCCATGTAC

C      L E G L T G T S L P K E R G S T R V H A -

CCTTAAATAACGTCAACAGAGTGTGTCAGGTTTACATCAGAACAATGTGGAATTAGTGA
361 -----+-----+-----+-----+-----+-----+-----+-----+ 420
GGAATTTATTGCAGTTGTCTCACGACGTCCAAAATGTAGTCTTGTACACCTTAATCACT

C      L N N V N R V L Q V L H Q N N V E L V N -

ATATAGGGGGAACGTGACATTGTGGATGGAATCACAACCTGACTTTGGGGTTACTTTGGA
421 -----+-----+-----+-----+-----+-----+-----+-----+ 480
TATATCCCCCTTGACTGTAACACCTACCTTTAGTGTGTTGACTGAAACCCCAATGAAACCT

C      I G G T D I V D G N H K L T L G L L W S -

GCATCATTTTGCAGTGGCAGGTGAAAGATGTCATGAAGGATGTCATGTCGGACCTGCAGC
481 -----+-----+-----+-----+-----+-----+-----+-----+ 540
CGTAGTAAACGTGACCGTCCACTTTCTACAGTACTTCCTACAGTACAGCCTGGACGTCG

C      I I L H W Q V K D V M K D V M S D L Q Q -

AGACGAACAQGTGAGAAGATCCTGCTCAGCTGGGTGCGTCAGACCACCAGGCCCTACAGCC
541 -----+-----+-----+-----+-----+-----+-----+-----+ 600
TCTGCTTGTCAGTCTTCTAGGACGAGTCGACCCACGCGAGTCTGGTGGTCCGGGATGTCGG

C      T N S E K I L L S W V R Q T T R P Y S Q -

AAGTCAACGTCCTCAACTTCACCACCAGCTGGACAGATGGACTCGCCTTTAATGCTGTCC
601 -----+-----+-----+-----+-----+-----+-----+-----+ 660
TTCAGTTGCAGGAGTTGAAGTGGTGGTCGACCTGTCTACCTGAGCGGAAATTACGACAGG

C      V N V L N F T T S W T D G L A F N A V L -

TCCACCGACATAAACCTGATCTCTTCAGCTGGGATAAAGTTGTCAAATGTCACCAATTG
661 -----+-----+-----+-----+-----+-----+-----+-----+ 720
AGGTGGCTGTATTTGGACTAGAGAAGTCGACCCTATTTCAACAGTTTACAGTGGTTAAC

C      H R H K P D L F S W D K V V K M S P I E -

```

Figure 8

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Figure 8 cont ...

Figure 9

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L M E L Q K K Q L Q Q L S S W L A L T E E R I Q K M E>
 1370 1380 1390 1400 1410 1420 1430 1440
 AGCCTCCCGC TGGGTGATGA CCTGCCCTCC CTGCAGAAGC TGCTTCAAGA ACATAAAAGT TTGCAAAATG ACCTTGAAGC
 S L P L G D D L P S L Q K L L Q E H K S L Q N D L E A>
 1450 1460 1470 1480 1490 1500 1510 1520
 TGAACAGGTG AAGGTAAATT CCTTAACCTCA CATGGTGGTG ATTGTGGATG AAAACAGTGG GGAGAGTGCC ACAGCTCTTC
 E Q V K V N S L T H M V V I V D E N S G E S A T A L>
 1530 1540 1550 1560 1570 1580 1590 1600
 TGGAAGATCA GTTACAGAAA CTGGGTGAGC GCTGGACAGC TGTATGCCGC TGGACTGAAG AACGTTGGAA CAGGTTGCAA
 L E D Q L Q K L G E R W T A V C R W T E E R W N R L Q>
 1610 1620 1630 1640 1650 1660 1670 1680
 GAAATCAGTA TTCTGTGGCA GGAATTATG GAAGAGCAGT GTCTGTGGGA GGCTTGGCTC ACCGAAAAGG AAGAGGCTTT
 E I S I L W Q E L L E E Q C L L E A W L T E K E E A L>
 1690 1700 1710 1720 1730 1740 1750 1760
 GGATAAAGTT CAAACCAGCA ACTTTAAAGA CCAGAAGGAA CTAAGTGTCA GTGTCCGGCG TCTGGCTATA TTGAAGGAAG
 D K V Q T S N F K D Q K E L S V S V R R L A I L K E>
 1770 1780 1790 1800 1810 1820 1830 1840
 ACATGGAAAT GAAGAGGCAG ACTCTGGATC AACTGAGTGA GATTGGCCAG GATGTGGGCC AATTACTCAG TAATCCCAAG
 D M E M K R Q T L D Q L S E I G Q D V G Q L L S N P K>
 1850 1860 1870 1880 1890 1900 1910 1920
 GCATCTAAGA AGATGAACAG TGACTCTGAG GAGCTAACAC AGAGATGGGA TTCTCTGGTT CAGAGACTCG AAGACTCTTC
 A S K K M N S D S E E L T Q R W D S L V Q R L E D S S>
 1930 1940 1950 1960 1970 1980 1990 2000
 TAACCAGGTG ACTCAGGCGG TAGCGAAGCT CGGCATGTCC CAGATTCCAC AGAAGGACCT ATTGGAGACC GTTCATGTGA
 N Q V T Q A V A K L G M S Q I P Q K D L L E T V H V>
 2010 2020 2030 2040 2050 2060 2070 2080
 GAGAACAGG GATGGTGAAG AAGCCCAAGC AGGAATGCC TCCTCCGTTA ACAAAGGCTG AGCATGCTAT GCAAAAGAGA
 R E Q G M V K K P K Q E L P P P L T K A E H A M Q K R>
 2090 2100 2110 2120 2130 2140 2150 2160
 TCAACCACCG AATTGGGAGA AAACCTGCAA GAATTAAGAG ACTTAACCTCA AGAAATGGAA GTACATGCTG AAAAAGTCAA
 S T T E L G E N L Q E L R D L T Q E M E V H A E K L K>
 2170 2180 2190 2200 2210 2220 2230 2240
 ATGGCTGAAT AGAACTGAAT TGGAGATGCT TTCAGATAAA AGTCTGAGTT TACCTGAAAG GGATAAAATT TCAGAAAGCT
 W L N R T E L E M L S D K S L S L P E R D K I S E S>
 2250 2260 2270 2280 2290 2300 2310 2320
 TAAGGACTGT AAATATGACA TGAATAAGA TTTGCAGAGA GGTGCCTACC ACCCTGAAGG AATGCATCCA GGAGCCAGT
 L R T V N M T W N K I C R E V P T T L K E C I Q E P S>
 2330 2340 2350 2360 2370 2380 2390 2400
 TCTGTTTCAC AGACAAGGAT TGCTGCTCAT CCTAATGTCC AAAAGGTGGT GCTAGTATCA TCTGCGTCAG ATATTCCTGT
 S V S Q T R I A A H P N V Q K V V L V S S A S D I P V>
 2410 2420 2430 2440 2450 2460 2470 2480
 TCAGTCTCAT CGTACTTCGG AAATTTCAAT TCCTGCTGAT CTTGATAAAA CTATAACAGA ACTAGCCGAC TGGCTGGTAT
 Q S H R T S E I S I P A D L D K T I T E L A D W L V>
 2490 2500 2510 2520 2530 2540 2550 2560
 TAATCGACCA GATGCTGAAG TCCAACATTG TCACTGTTGG GGATGTAGAA GAGATCAATA AGACCGTTTC CCGAATGAAA
 L I D Q M L K S N I V T V G D V E E I N K T V S R M K>
 2570 2580 2590 2600 2610 2620 2630 2640
 ATTACAAAGG CTGACTTAGA ACAGCGCCAT CCTCAGCTGG ATTATGTTTT TACATTGGCA CAGAAATTTGA AAAATAAAGC
 I T K A D L E Q R H P Q L D Y V F T L A Q N L K N K A>
 2650 2660 2670 2680 2690 2700 2710 2720
 TTCCAGTTCA GATATGAGAA CAGCAATTAC AGAAAAATTG GAAAGGTGCA AGAACCAGTG GGATGGCACC CAGCATGGCG

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S S S D M R T A I T E K L E R V K N Q W D G T Q H G>
 2730 2740 2750 2760 2770 2780 2790 2800
 TTGAGCTAAG ACAGCAGCAG CTTGAGGACA TGATTATTGA CAGTCTTCAG TGGGATGACC ATAGGGGAGGA GACTGAAGAA
 V E L R Q Q Q L E D M I I D S L Q W D D H R E E T E E>
 2810 2820 2830 2840 2850 2860 2870 2880
 CTGATGAGAA AATATGAGGC TCGACTCTAT ATTCTTCAGC AAGCCCAGC GGATCCACTC ACCAAACAAA TTTCTGATAA
 L M R K Y E A R L Y I L Q Q A R R D P L T K Q I S D N>
 2890 2900 2910 2920 2930 2940 2950 2960
 CCAAATACTG CTTCAAGAAC TGGGTCCTGG AGATGGTATC GTCATGGCGT TCGATAACGT CCTGCAGAAA CTCTGGAGG
 Q I L L Q E L G P G D G I V M A F D N V L Q K L L E>
 2970 2980 2990 3000 3010 3020 3030 3040
 AATATGGGAG TGATGACACA AGGAATGTGA AAGAAACCAC AGAGTACTTA AAAACATCAT GGATCAATCT CAAACAAAGT
 E Y G S D D T R N V K E T T E Y L K T S W I N L K Q S>
 3050 3060 3070 3080 3090 3100 3110 3120
 ATTGCTGACA GACAGAACGC CTTGGAGGCT GAGTGGAGGA CGGTGCAGGC CTCTCGCAGA GATCTGGAAG ACTTCTTGAA
 I A D R Q N A L E A E W R T V Q A S R R D L E N F L K>
 3130 3140 3150 3160 3170 3180 3190 3200
 GTGGATCCAA GAAGCAGAGA CCACACTGAA TGTGCTTGTG GATGCCTCTC ATCGGAGAGAA TGCTCTTCAG GATAGTATCT
 W I Q E A E T T V N V L V D A S H R E N A L Q D S I>
 3210 3220 3230 3240 3250 3260 3270 3280
 TGGCCAGGGA ACTCAAACAG CAGATGCAGG ACATCCAGGC AGAAATTGAT GCCCACAATG ACATATTTAA AAGCATTGAC
 L A R E L K Q Q M Q D I Q A E I D A H N D I F K S I D>
 3290 3300 3310 3320 3330 3340 3350 3360
 GGAAACAGGC AGAAGATGGT AAAAGCTTTG GGAAATTCTG AAGAGGCTAC TATGCTTCAA CATCGACTGG ATGATATGAA
 G N R Q K M V K A L G N S E E A T M L Q H R L D D M N>
 3370 3380 3390 3400 3410 3420 3430 3440
 CCAAAGATGG AATGACTTAA AAGCAAAATC TGCTAGCATC AGGGCCCAT TGGAGGCCAG CGCTGAGAAG TGGAACAGGT
 Q R W N D L K A K S A S I R A H L E A S A E K W N R>
 3450 3460 3470 3480 3490 3500 3510 3520
 TGCTGATGTC CTTAGAAGAA CTGATCAAAT GGCTGAATAT GAAAGATGAA GAGCTTAAGA AACAAATGCC TATTGGAGGA
 L L M S L E E L I K W L N M K D E E L K K Q M P I G G>
 3530 3540 3550 3560 3570 3580 3590 3600
 GATGTTCCAG CTTACAGCT CCAGTATGAC CATTGTAAGG CCCTGAGACG GGAGTTAAAG GAGAAAGAAT ATTCTGTCT
 D V P A L Q L Q Y D H C K A L R R E L K E K E Y S V L>
 3610 3620 3630 3640 3650 3660 3670 3680
 GAATGCTGTC GACCAGGCC GAGTTTCTT GGCTGATCAG CCAATTGAGG CCCCTGAAGA GCCAAGAAGA AACCTACAAT
 N A V D Q A R V F L A D Q P I E A P E E P R R N L Q>
 3690 3700 3710 3720 3730 3740 3750 3760
 CAAAAACAGA ATTAACCTCT GAGGAGAGAG CCCAAAAGAT TGCCAAAGCC ATGCGCAAAC AGTCTTCTGA AGTCAAAGAA
 S K T E L T P E E R A Q K I A K A M R K Q S S E V K E>
 3770 3780 3790 3800 3810 3820 3830 3840
 AAATGGGAAA GTCTAAATGC TGTAAGTAGC AATTGGCAAA AGCAAGTGGA CAAGGCATTG GAGAAACTCA GAGACCTGCA
 K W E S L N A V T S N W Q K Q V D K A L E K L R D L Q>
 3850 3860 3870 3880 3890 3900 3910 3920
 GGGAGCTATG GATGACCTGG ACGCTGACAT GAAGGAGGCA GAGTCCGTGC GGAATGGCTG GAAGCCCGTG GGAGACTTAC
 G A M D D L D A D M K E A E S V R N G W K P V G D L>
 3930 3940 3950 3960 3970 3980 3990 4000
 TCATTGACTC GCTGCAGGAT CACATTGAAA AAATCATGGC ATTTAGAGAA GAAATGACAC CAATCAACTT TAAAGTTAAA
 L I D S L Q D H I E K I M A F R E E I A P I N F K V K>
 4010 4020 4030 4040 4050 4060 4070 4080
 ACGGTGAATG ATTTATCCAG TCAGTGTCT CCACTTGACC TGCATCCCTC TCTAAAGATG TCTCGCCAGC TAGATGACCT

Figure 9 cont ...

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T V N D L S S Q L S P L D L H P S L K M S R Q L D D L>

4090 4100 4110 4120 4130 4140 4150 4160
TAATATGCGA TGGAAACTTT TACAGGTTTC TGTGGATGAT CGCCTTAAAC AGCTTCAGGA AGCCACACAGA GATTTTGGAC
N M R W K L L Q V S V D D R L K Q L Q E A H R D F G>

4170 4180 4190 4200 4210 4220 4230 4240
CATCCTCTCA GCATTTTCTC TCTACGTCAG TCCAGCTGCC GTGGCAAAGA TCCATTTCAC ATAATAAAGT GCCCTATTAC
P S S Q H F L S T S V Q L P W Q R S I S H N K V P Y Y>

4250 4260 4270 4280 4290 4300 4310 4320
ATCAACCATC AAACACAGAC CACCTGTTGG GACCATCCTA AAATGACCGA ACTCTTTCAA TCCCTTGCTG ACCTGAATAA
I N H Q T Q T T C W D H P K M T E L F Q S L A D L N N>

4330 4340 4350 4360 4370 4380 4390 4400
TGTACGTTTT TCTGCCTACC GTACAGCAAT CAAAATCCGA AGACTACAAA AAGCACTATG TTTGGATCTC TTAGAGTTGA
V R F S A Y R T A I K I R R L Q K A L C L D L L E L>

4410 4420 4430 4440 4450 4460 4470 4480
GTACAACAAA TGAAATTTTC AAACAGCACA AGTTGAACCA AAATGACCGA CTCCTCAGTG TTCCAGATGT CATCAACTGT
S T T N E I F K Q H K L N Q N D Q L L S V P D V I N C>

4490 4500 4510 4520 4530 4540 4550 4560
CTGACAACAA CTTATGATGG ACTTGAGCAA ATGCATAAGG ACCTGGTCAA CGTTCCACTC TGTGTTGATA TGTGTTCTCA
L T T T Y D G L E Q M H K D L V N V P L C V D M C L N>

4570 4580 4590 4600 4610 4620 4630 4640
TTGGTTGCTC AATGTCTATG ACACGGGTG AACTGGAAAA ATTAGAGTGC AGAGTCTGAA GATTGGATTA ATGTCTCTCT
W L L N V Y D T G R T G K I R V Q S L K I G L M S L>

4650 4660 4670 4680 4690 4700 4710 4720
CCAAAGGTCT CTTGGAAGAA AAATACAGAT ATCTCTTTAA GGAAGTTGCG GGGCCGACAG AAATGTGTGA CCAGAGGCAG
S K G L L E E K Y R Y L F K E V A G P T E M C D Q R Q>

4730 4740 4750 4760 4770 4780 4790 4800
CTGGGCCTGT TACTTCATGA TGCCATCCAG ATCCCCCGGC AGCTAGGTGA AGTAGCAGCT TTTGGAGGCA GTAATATTGA
L G L L L H D A I Q I P R Q L G E V A A F G G S N I E>

4810 4820 4830 4840 4850 4860 4870 4880
GCCTAGTGTT CGCAGCTGCT TCCAACAGAA TAACAATAAA CCAGAAATAA GTGTGAAAGA GTTTATAGAT TGGATGCAAT
P S V R S C F Q Q N N N K P E I S V K E F I D W M H>

4890 4900 4910 4920 4930 4940 4950 4960
TGGAACCACA GTCCATGGTT TGGCTCCAG TTTTACATCG AGTGGCAGCA GCGGAGACTG CAAAACATCA GGCCAAATGC
L E P Q S M V W L P V L H R V A A A E T A K H Q A K C>

4970 4980 4990 5000 5010 5020 5030 5040
AACATCTGTA AAGAATGTCC AATTGTGGGG TTCAGGTATA GAAGCCTTAA GCATTTTAAC TATGATGTCT GCCAGAGTTG
N I C K E C P I V G F R Y R S L K H F N Y D V C Q S C>

5050 5060 5070 5080 5090 5100 5110 5120
TTTCTTTTCG GGTGGAACAG CAAAAGGTCA CAAATTACAT TACCCAATGG TGGAATATTG TATACCTACA ACATCTGGGG
F F S G R T A K G H K L H Y P M V E Y C I P T T S G>

5130 5140 5150 5160 5170 5180 5190 5200
AAGATGTACG AGACTTCACA AAGGTACTTA AGAACAAGTT CAGGTGCAAG AAGTACTTTG CCAAACACCC TCGACTTGGT
E D V R D F T K V L K N K F R S K K Y F A K H P R L G>

5210 5220 5230 5240 5250 5260 5270 5280
TACCTGCTCG TCCAGACAGT TCTTGAAGGT GACAACCTAG AGACTCCTAT CACACTCATC AGTATGTGGC CAGAGCATA
Y L P V Q T V L E G D N L E T P I T L I S M W P E H Y>

5290 5300 5310 5320 5330 5340 5350 5360
TGACCCCTCA CAATCTCTC AACTGTTTCA TGATGACACC CATTCAAGAA TAGAACAATA TGCCACACGA CTGGCCCGAGA
D P S Q S P Q L F H D D T H S R I E Q Y A T R L A Q>

5370 5380 5390 5400 5410 5420 5430 5440
TGGAAAGGAC TAATGGGTCT TTTCTCACTG ATAGCAGCTC CACCACAGGA AGTGTGGAAG ACGAGCACGC CCTCATCCAG

Figure 9 cont ...

M E R T N G S F L T D S S S T T G S V E D E H A L I Q>
5450 5460 5470 5480 5490 5500 5510 5520
CAGTATTGCC AAACACTCGG AGGAGAGTCC CCAGTGAGCC AGCCGCAGAG CCCAGCTCAG ATCCTGAAGT CAGTAGAGAG
Q Y C Q T L G G E S P V S Q P Q S P A Q I L K S V E R>
5530 5540 5550 5560 5570 5580 5590 5600
GGAAGAACGT GGAGAACTGG AGAGGATCAT TGCTGACCTG GAGGAAGAAC AAAGAAATCT ACAGGTGGAG TATGAGCAGC
E E R G E L E R I I A D L E E E Q R N L Q V E Y E Q>
5610 5620 5630 5640 5650 5660 5670 5680
TGAAGGACCA GCACCTCCGA AGGGGGCTCC CTGTCGGTTC ACCGCCAGAG TCGATTATAT CTCCCCATCA CACGTCTGAG
L K D Q H L R R G L P V G S P P E S I I S P H H T S E>
5690 5700 5710 5720 5730 5740 5750 5760
GATTCAGAAC TTATAGCAGA AGCAAACTC CTCAGGCAGC ACAAAGGTCT GCTGGAGGCT AGGATGCAGA TTTTAGAAGA
D S E L I A E A K L L R Q H K G R L E A R M Q I L E D>
5770 5780 5790 5800 5810 5820 5830 5840
TCACAATAAA CAGCTGGAGT CTCAGCTCCA CCGCCTCCGA CAGCTGCTGG AGCAGCCTGA ATCTGATTCC CGAATCAATG
H N K Q L E S Q L H R L R Q L L E Q P E S D S R I N>
5850 5860 5870 5880 5890 5900 5910 5920
GTGTTTCCCC ATGGGCTTCT CCTCAGCATT CTGCACTGAG CTA CTGCTT GATCCAGATG CCTCCGGCCC ACAGTTCCAC
G V S P W A S P Q H S A L S Y S L D P D A S G P Q F H>
5930 5940 5950 5960 5970 5980 5990 6000
CAGGCAGCGG GAGAGGACCT GCTGGCCCCA CCGCACGACA CCAGCACGGA TCTCACGGAG GTCATGGAGC AGATTACAG
Q A A G E D L L A P P H D T S T D L T E V M E Q I H S>
6010 6020 6030 6040 6050
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T F P S C C P N V P S R P Q A M *>

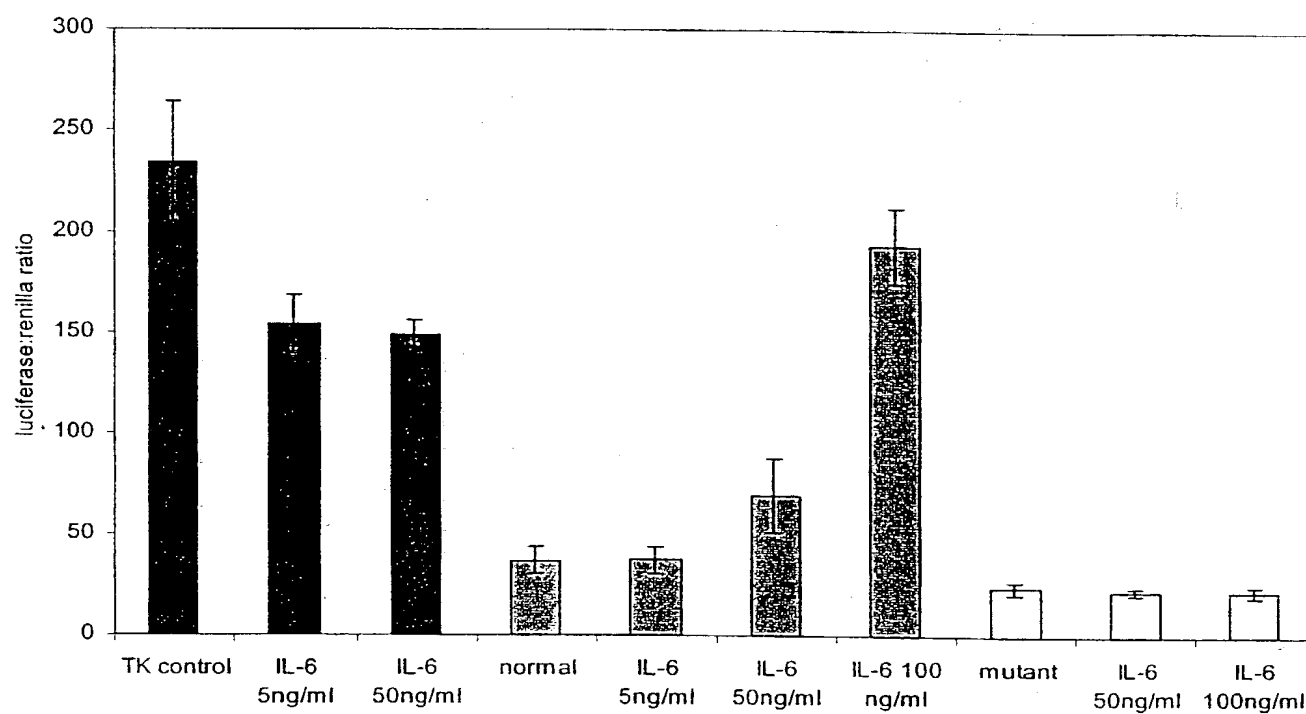


Figure 10

INVENTOR DECLARATION

Attorney Docket No.

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled
, the specification of which

(check one) ☐ is attached hereto.

☒ was filed on _____ as Application Serial No. _____
(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed:

| Prior Foreign Application(s) | | | Priority Claimed | |
|------------------------------|-----------------------|------------------------|-------------------------------------|--------------------------|
| <u>9923423.9</u> | <u>United Kingdom</u> | <u>4 October 1999</u> | <input checked="" type="checkbox"/> | <input type="checkbox"/> |
| (Number) | (Country) | (Day/Month/Year Filed) | Yes | No |
| _____ | _____ | _____ | <input checked="" type="checkbox"/> | <input type="checkbox"/> |
| (Number) | (Country) | (Day/Month/Year Filed) | Yes | No |
| _____ | _____ | _____ | <input type="checkbox"/> | <input type="checkbox"/> |
| (Number) | (Country) | (Day/Month/Year Filed) | Yes | No |

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below:

| | |
|--------------------------|---------------|
| _____ | _____ |
| (Application Serial No.) | (Filing Date) |
| _____ | _____ |
| (Application Serial No.) | (Filing Date) |

I hereby claim the benefit under Title 35, United States Code § 120 of any United States application(s), or § 365(b) of any PCT international application designating the United States of America, listed below and insofar as the subject matter of each of the claims of this application is not disclosed in the prior U.S. or PCT international application in the manner provided by the first paragraph of Title 35, U.S.C. § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

| | | |
|--------------------------|-----------------------|--------------------------------|
| <u>PCT/GB 00/03800</u> | <u>4 October 2000</u> | _____ |
| (Application Serial No.) | (Filing Date) | (Status) |
| _____ | _____ | (patented, pending, abandoned) |
| (Application Serial No.) | (Filing Date) | (Status) |
| _____ | _____ | (patented, pending, abandoned) |

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Application No.

Docket No.

Please direct all correspondence regarding this application to the following:

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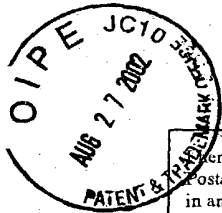
For: Isis Innovation Limited

By: 

Dated: 15 - MAY - 2002

Printed Name: 

Mr. T. Hockaday
Executive Director
Isis Innovation Ltd



Rec'd PCT/PTO 27 AUG 2002
10/089928

N.82592A

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as Express Mail, Airbill No. EU110398085US in an envelope address to: Commissioner for Patents, Washington, DC 20231, on the date shown below

Dated: 8/27/02 Signature: Staci V. Harris
(By: Staci Harris)

Docket No.

(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Isis Innovation Limited

Customer No.:

Application No.:

Group Art Unit:

Filed:

Examiner:

For: UTROPHIN GENE PROMOTER

POWER OF ATTORNEY

Commissioner for Patents
Washington, DC 20231

Dear Sir:

Isis Innovation Limited, assignee of the entire right, title and interest in the above-identified application by assignment dated, submitted herewith, hereby appoints the members of the firm of Fulbright & Jaworski L.L.P., a firm composed of:

22

| | | | | | |
|---------------------|---------------|---------------------|-----------------|------------------------|---------------|
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| Alberto Q. Amatong | <u>41,580</u> | Michael S. McCoy | <u>46,913</u> | Jan K. Simpson | <u>33,283</u> |
| Ronald G. Bliss | <u>28,691</u> | John M. Mings | <u>35,955</u> | Melissa L. Sistrunk | <u>45,579</u> |
| Gino Catena | <u>45,546</u> | Thomas D. Paul | <u>32,714</u> | Edward D. Steakley | <u>47,964</u> |
| Paul L. Deverter II | <u>19,747</u> | Jayne C. Piana | <u>P-48,424</u> | William A. Stout | <u>18,773</u> |
| Mark L. Delflache | <u>28,942</u> | Doak C. Procter, IV | <u>P-48,705</u> | Charles B. Walker, Jr. | <u>43,231</u> |
| David L. Fox | <u>40,612</u> | James W. Repass | <u>30,487</u> | Richard S. Zembek | <u>43,306</u> |
| Eric B. Hall | <u>46,751</u> | | | | |

as its attorneys with full power of substitution to prosecute this application and to transact all business in the Patent and Trademark Office in connection therewith.

The assignee certifies that it has reviewed the assignment and to the best of the assignee's knowledge and belief, title is in the assignee.

N.82592A

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JC13 Rec'd PCT/PTO 04 APR 2002

SEQUENCE LISTING

<110> Burton, Edward
Tinsley, Jonathan
Davies, Kay

<120> Utrophin Gene Promoter

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| gcc tta aac aat gtc aac cga gtg cta cag gtt tta cat cag aac aat | 337 |
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| Lys Leu Thr Leu Gly Leu Leu Trp Ser Ile Ile Leu His Trp Gln Val | |
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| Thr Trp Asn Lys Ile Cys Arg Glu Val Pro Thr Thr Leu Lys Glu Cys | |
| 750 755 760 765 | |
| atc cag gag ccc agt tct gtt tca cag aca agg att gct gct cat cct | 2353 |
| Ile Gln Glu Pro Ser Ser Val Ser Gln Thr Arg Ile Ala Ala His Pro | |

| 770 | | | | | | | | | | 775 | | | | | | | | | | 780 | | | | | | | | | | |
|---|------|--|--|--|--|--|--|--|--|-----|--|--|--|--|--|--|--|--|--|-----|--|--|--|--|--|--|--|--|--|--|
| aat gtc caa aag gtg gtg cta gta tca tct gcg tca gat att cct gtt | 2401 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Asn Val Gln Lys Val Val Leu Val Ser Ser Ala Ser Asp Ile Pro Val | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 785 790 795 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| cag tct cat cgt act tcg gaa att tca att cct gct gat ctt gat aaa | 2449 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Gln Ser His Arg Thr Ser Glu Ile Ser Ile Pro Ala Asp Leu Asp Lys | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 800 805 810 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| act ata aca gaa cta gcc gac tgg ctg gta tta atc gac cag atg ctg | 2497 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Thr Ile Thr Glu Leu Ala Asp Trp Leu Val Leu Ile Asp Gln Met Leu | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 815 820 825 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| aag tcc aac att gtc act gtt ggg gat gta gaa gag atc aat aag acc | 2545 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lys Ser Asn Ile Val Thr Val Gly Asp Val Glu Glu Ile Asn Lys Thr | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 830 835 840 845 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| gtt tcc cga atg aaa att aca aag gct gac tta gaa cag cgc cat cct | 2593 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Val Ser Arg Met Lys Ile Thr Lys Ala Asp Leu Glu Gln Arg His Pro | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 850 855 860 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| cag ctg gat tat gtt ttt aca ttg gca cag aat ttg aaa aat aaa gct | 2641 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Gln Leu Asp Tyr Val Phe Thr Leu Ala Gln Asn Leu Lys Asn Lys Ala | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 865 870 875 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| tcc agt tca gat atg aga aca gca att aca gaa aaa ttg gaa agg gtc | 2689 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Ser Ser Ser Asp Met Arg Thr Ala Ile Thr Glu Lys Leu Glu Arg Val | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 880 885 890 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| aag aac cag tgg gat ggc acc cag cat ggc gtt gag cta aga cag cag | 2737 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lys Asn Gln Trp Asp Gly Thr Gln His Gly Val Glu Leu Arg Gln Gln | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 895 900 905 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| cag ctt gag gac atg att att gac agt ctt cag tgg gat gac cat agg | 2785 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Gln Leu Glu Asp Met Ile Ile Asp Ser Leu Gln Trp Asp Asp His Arg | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 910 915 920 925 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| gag gag act gaa gaa ctg atg aga aaa tat gag gct cga ctc tat att | 2833 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Glu Glu Thr Glu Glu Leu Met Arg Lys Tyr Glu Ala Arg Leu Tyr Ile | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 930 935 940 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| ctt cag caa gcc cga cgg gat cca ctc acc aaa caa att tct gat aac | 2881 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Leu Gln Gln Ala Arg Arg Asp Pro Leu Thr Lys Gln Ile Ser Asp Asn | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 945 950 955 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| caa ata ctg ctt caa gaa ctg ggt cct gga gat ggt atc gtc atg gcg | 2929 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Gln Ile Leu Leu Gln Glu Leu Gly Pro Gly Asp Gly Ile Val Met Ala | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 960 965 970 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| ttc gat aac gtc ctg cag aaa ctc ctg gag gaa tat ggg agt gat gac | 2977 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Phe Asp Asn Val Leu Gln Lys Leu Leu Glu Glu Tyr Gly Ser Asp Asp | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 975 980 985 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| aca agg aat gtg aaa gaa acc aca gag tac tta aaa aca tca tgg atc | 3025 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Thr Arg Asn Val Lys Glu Thr Thr Glu Tyr Leu Lys Thr Ser Trp Ile | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 990 995 1000 1005 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| aat ctc aaa caa agt att gct gac aga cag aac gcc ttg gag gct gag | 3073 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Asn Leu Lys Gln Ser Ile Ala Asp Arg Gln Asn Ala Leu Glu Ala Glu | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1010 1015 1020 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

| | |
|---|------|
| tgg agg acg gtg cag gcc tct cgc aga gat ctg gaa aac ttc ctg aag | 3121 |
| Trp Arg Thr Val Gln Ala Ser Arg Arg Asp Leu Glu Asn Phe Leu Lys | |
| 1025 1030 1035 | |
| tgg atc caa gaa gca gag acc aca gtg aat gtg ctt gtg gat gcc tct | 3169 |
| Trp Ile Gln Glu Ala Glu Thr Thr Val Asn Val Leu Val Asp Ala Ser | |
| 1040 1045 1050 | |
| cat cgg gag aat gct ctt cag gat agt atc ttg gcc agg gaa ctc aaa | 3217 |
| His Arg Glu Asn Ala Leu Gln Asp Ser Ile Leu Ala Arg Glu Leu Lys | |
| 1055 1060 1065 | |
| cag cag atg cag gac atc cag gca gaa att gat gcc cac aat gac ata | 3265 |
| Gln Gln Met Gln Asp Ile Gln Ala Glu Ile Asp Ala His Asn Asp Ile | |
| 1070 1075 1080 1085 | |
| ttt aaa agc att gac gga aac agg cag aag atg gta aaa gct ttg gga | 3313 |
| Phe Lys Ser Ile Asp Gly Asn Arg Gln Lys Met Val Lys Ala Leu Gly | |
| 1090 1095 1100 | |
| aat tct gaa gag gct act atg ctt caa cat cga ctg gat gat atg aac | 3361 |
| Asn Ser Glu Glu Ala Thr Met Leu Gln His Arg Leu Asp Asp Met Asn | |
| 1105 1110 1115 | |
| caa aga tgg aat gac tta aaa gca aaa tct gct agc atc agg gcc cat | 3409 |
| Gln Arg Trp Asn Asp Leu Lys Ala Lys Ser Ala Ser Ile Arg Ala His | |
| 1120 1125 1130 | |
| ttg gag gcc agc gct gag aag tgg aac agg ttg ctg atg tcc tta gaa | 3457 |
| Leu Glu Ala Ser Ala Glu Lys Trp Asn Arg Leu Leu Met Ser Leu Glu | |
| 1135 1140 1145 | |
| gaa ctg atc aaa tgg ctg aat atg aaa gat gaa gag ctt aag aaa caa | 3505 |
| Glu Leu Ile Lys Trp Leu Asn Met Lys Asp Glu Glu Leu Lys Lys Gln | |
| 1150 1155 1160 1165 | |
| atg cct att gga gga gat gtt cca gcc tta cag ctc cag tat gac cat | 3553 |
| Met Pro Ile Gly Gly Asp Val Pro Ala Leu Gln Leu Gln Tyr Asp His | |
| 1170 1175 1180 | |
| tgt aag gcc ctg aga cgg gag tta aag gag aaa gaa tat tct gtc ctg | 3601 |
| Cys Lys Ala Leu Arg Arg Glu Leu Lys Glu Lys Glu Tyr Ser Val Leu | |
| 1185 1190 1195 | |
| aat gct gtc gac cag gcc cga gtt ttc ttg gct gat cag cca att gag | 3649 |
| Asn Ala Val Asp Gln Ala Arg Val Phe Leu Ala Asp Gln Pro Ile Glu | |
| 1200 1205 1210 | |
| gcc cct gaa gag cca aga aga aac cta caa tca aaa aca gaa tta act | 3697 |
| Ala Pro Glu Glu Pro Arg Arg Asn Leu Gln Ser Lys Thr Glu Leu Thr | |
| 1215 1220 1225 | |
| cct gag gag aga gcc caa aag att gcc aaa gcc atg cgc aaa cag tct | 3745 |
| Pro Glu Glu Arg Ala Gln Lys Ile Ala Lys Ala Met Arg Lys Gln Ser | |
| 1230 1235 1240 1245 | |
| tct gaa gtc aaa gaa aaa tgg gaa agt cta aat gct gta act agc aat | 3793 |
| Ser Glu Val Lys Glu Lys Trp Glu Ser Leu Asn Ala Val Thr Ser Asn | |
| 1250 1255 1260 | |

tgg caa aag caa gtg gac aag gca ttg gag aaa ctc aga gac ctg cag 3841
 Trp Gln Lys Gln Val Asp Lys Ala Leu Glu Lys Leu Arg Asp Leu Gln
 1265 1270 1275

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 Gly Ala Met Asp Asp Leu Asp Ala Asp Met Lys Glu Ala Glu Ser Val
 1280 1285 1290

cgg aat ggc tgg aag ccc gtg gga gac tta ctc att gac tcg ctg cag 3937
 Arg Asn Gly Trp Lys Pro Val Gly Asp Leu Leu Ile Asp Ser Leu Gln
 1295 1300 1305

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 Asp His Ile Glu Lys Ile Met Ala Phe Arg Glu Glu Ile Ala Pro Ile
 1310 1315 1320 1325

aac ttt aaa gtt aaa acg gtg aat gat tta tcc agt cag ctg tct cca 4033
 Asn Phe Lys Val Lys Thr Val Asn Asp Leu Ser Ser Gln Leu Ser Pro
 1330 1335 1340

ctt gac ctg cat ccc tct cta aag atg tct cgc cag cta gat gac ctt 4081
 Leu Asp Leu His Pro Ser Leu Lys Met Ser Arg Gln Leu Asp Asp Leu
 1345 1350 1355

aat atg cga tgg aaa ctt tta cag gtt tct gtg gat gat cgc ctt aaa 4129
 Asn Met Arg Trp Lys Leu Leu Gln Val Ser Val Asp Asp Arg Leu Lys
 1360 1365 1370

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 Gln Leu Gln Glu Ala His Arg Asp Phe Gly Pro Ser Ser Gln His Phe
 1375 1380 1385

ctc tct acg tca gtc cag ctg ccg tgg caa aga tcc att tca cat aat 4225
 Leu Ser Thr Ser Val Gln Leu Pro Trp Gln Arg Ser Ile Ser His Asn
 1390 1395 1400 1405

aaa gtg ccc tat tac atc aac cat caa aca cag acc acc tgt tgg gac 4273
 Lys Val Pro Tyr Tyr Ile Asn His Gln Thr Gln Thr Thr Cys Trp Asp
 1410 1415 1420

cat cct aaa atg acc gaa ctc ttt caa tcc ctt gct gac ctg aat aat 4321
 His Pro Lys Met Thr Glu Leu Phe Gln Ser Leu Ala Asp Leu Asn Asn
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 Val Arg Phe Ser Ala Tyr Arg Thr Ala Ile Lys Ile Arg Arg Leu Gln
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 Lys Ala Leu Cys Leu Asp Leu Leu Glu Leu Ser Thr Thr Asn Glu Ile
 1455 1460 1465

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 Phe Lys Gln His Lys Leu Asn Gln Asn Asp Gln Leu Leu Ser Val Pro
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gat gtc atc aac tgt ctg aca aca act tat gat gga ctt gag caa atg 4513
 Asp Val Ile Asn Cys Leu Thr Thr Thr Tyr Asp Gly Leu Glu Gln Met
 1490 1495 1500

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His Lys Asp Leu Val Asn Val Pro Leu Cys Val Asp Met Cys Leu Asn
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 Trp Leu Leu Asn Val Tyr Asp Thr Gly Arg Thr Gly Lys Ile Arg Val
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 Gln Ser Leu Lys Ile Gly Leu Met Ser Leu Ser Lys Gly Leu Leu Glu
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gaa aaa tac aga tat ctc ttt aag gaa gtt gcg ggg ccg aca gaa atg 4705
 Glu Lys Tyr Arg Tyr Leu Phe Lys Glu Val Ala Gly Pro Thr Glu Met
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tgt gac cag agg cag ctg ggc ctg tta ctt cat gat gcc atc cag atc 4753
 Cys Asp Gln Arg Gln Leu Gly Leu Leu Leu His Asp Ala Ile Gln Ile
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 Pro Arg Gln Leu Gly Glu Val Ala Ala Phe Gly Gly Ser Asn Ile Glu
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cct agt gtt cgc agc tgc ttc caa cag aat aac aat aaa cca gaa ata 4849
 Pro Ser Val Arg Ser Cys Phe Gln Gln Asn Asn Asn Lys Pro Glu Ile
 1600 1605 1610

agt gtg aaa gag ttt ata gat tgg atg cat ttg gaa cca cag tcc atg 4897
 Ser Val Lys Glu Phe Ile Asp Trp Met His Leu Glu Pro Gln Ser Met
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 Val Trp Leu Pro Val Leu His Arg Val Ala Ala Ala Glu Thr Ala Lys
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 His Gln Ala Lys Cys Asn Ile Cys Lys Glu Cys Pro Ile Val Gly Phe
 1650 1655 1660

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 Arg Tyr Arg Ser Leu Lys His Phe Asn Tyr Asp Val Cys Gln Ser Cys
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 Phe Phe Ser Gly Arg Thr Ala Lys Gly His Lys Leu His Tyr Pro Met
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 Val Glu Tyr Cys Ile Pro Thr Thr Ser Gly Glu Asp Val Arg Asp Phe
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 Thr Lys Val Leu Lys Asn Lys Phe Arg Ser Lys Lys Tyr Phe Ala Lys
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cac cct cga ctt ggt tac ctg cct gtc cag aca gtt ctt gaa ggt gac 5233
 His Pro Arg Leu Gly Tyr Leu Pro Val Gln Thr Val Leu Glu Gly Asp
 1730 1735 1740

aac tta gag act cct atc aca ctc atc agt atg tgg cca gag cac tat 5281
 Asn Leu Glu Thr Pro Ile Thr Leu Ile Ser Met Trp Pro Glu His Tyr

| 1745 | 1750 | 1755 | |
|---|------|------|------|
| gac ccc tca caa tct cct caa ctg ttt cat gat gac acc cat tca aga Asp Pro Ser Gln Ser Pro Gln Leu Phe His Asp Asp Thr His Ser Arg 1760 1765 1770 | | | 5329 |
| ata gaa caa tat gcc aca cga ctg gcc cag atg gaa agg act aat ggg Ile Glu Gln Tyr Ala Thr Arg Leu Ala Gln Met Glu Arg Thr Asn Gly 1775 1780 1785 | | | 5377 |
| tct ttt ctc act gat agc agc tcc acc aca gga agt gtg gaa gac gag Ser Phe Leu Thr Asp Ser Ser Ser Thr Thr Gly Ser Val Glu Asp Glu 1790 1795 1800 1805 | | | 5425 |
| cac gcc ctc atc cag cag tat tgc caa aca ctc gga gga gag tcc cca His Ala Leu Ile Gln Gln Tyr Cys Gln Thr Leu Gly Gly Glu Ser Pro 1810 1815 1820 | | | 5473 |
| gtg agc cag ccg cag agc cca gct cag atc ctg aag tca gta gag agg Val Ser Gln Pro Gln Ser Pro Ala Gln Ile Leu Lys Ser Val Glu Arg 1825 1830 1835 | | | 5521 |
| gaa gaa cgt gga gaa ctg gag agg atc att gct gac ctg gag gaa gaa Glu Glu Arg Gly Glu Leu Glu Arg Ile Ile Ala Asp Leu Glu Glu Glu 1840 1845 1850 | | | 5569 |
| caa aga aat cta cag gtg gag tat gag cag ctg aag gac cag cac ctc Gln Arg Asn Leu Gln Val Glu Tyr Glu Gln Leu Lys Asp Gln His Leu 1855 1860 1865 | | | 5617 |
| cga agg ggg ctc cct gtc ggt tca ccg cca gag tcg att ata tct ccc Arg Arg Gly Leu Pro Val Gly Ser Pro Pro Glu Ser Ile Ile Ser Pro 1870 1875 1880 1885 | | | 5665 |
| cat cac acg tct gag gat tca gaa ctt ata gca gaa gca aaa ctc ctc His His Thr Ser Glu Asp Ser Glu Leu Ile Ala Glu Ala Lys Leu Leu 1890 1895 1900 | | | 5713 |
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| gag cag cct gaa tct gat tcc cga atc aat ggt gtt tcc cca tgg gct Glu Gln Pro Glu Ser Asp Ser Arg Ile Asn Gly Val Ser Pro Trp Ala 1935 1940 1945 | | | 5857 |
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| ggc cca cag ttc cac cag gca gcg gga gag gac ctg ctg gcc cca ccg Gly Pro Gln Phe His Gln Ala Ala Gly Glu Asp Leu Leu Ala Pro Pro 1970 1975 1980 | | | 5953 |
| cac gac acc agc acg gat ctc acg gag gtc atg gag cag att cac agc His Asp Thr Ser Thr Asp Leu Thr Glu Val Met Glu Gln Ile His Ser 1985 1990 1995 | | | 6001 |

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<210> 9
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 <213> Artificial Sequence

<220>
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 sequence of a utrophin B isoform "minigene"

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 Asp Leu Pro Gly His Val Ala Leu Gln Ala Cys Lys Arg Leu Pro Asp
 20 25 30
 Glu His Asn Asp Val Gln Lys Lys Thr Phe Thr Lys Trp Ile Asn Ala
 35 40 45
 Arg Phe Ser Lys Ser Gly Lys Pro Pro Ile Ser Asp Met Phe Ser Asp
 50 55 60
 Leu Lys Asp Gly Arg Lys Leu Leu Asp Leu Leu Glu Gly Leu Thr Gly
 65 70 75 80
 Thr Ser Leu Pro Lys Glu Arg Gly Ser Thr Arg Val His Ala Leu Asn
 85 90 95
 Asn Val Asn Arg Val Leu Gln Val Leu His Gln Asn Asn Val Asp Leu
 100 105 110
 Val Asn Ile Gly Gly Thr Asp Ile Val Ala Gly Asn Pro Lys Leu Thr
 115 120 125
 Leu Gly Leu Leu Trp Ser Ile Ile Leu His Trp Gln Val Lys Asp Val
 130 135 140
 Met Lys Asp Ile Met Ser Asp Leu Gln Gln Thr Asn Ser Glu Lys Ile
 145 150 155 160
 Leu Leu Ser Trp Val Arg Gln Thr Thr Arg Pro Tyr Ser Gln Val Asn
 165 170 175
 Val Leu Asn Phe Thr Thr Ser Trp Thr Asp Gly Leu Ala Phe Asn Ala
 180 185 190
 Val Leu His Arg His Lys Pro Asp Leu Phe Asp Trp Asp Glu Met Val
 195 200 205
 Lys Met Ser Pro Ile Glu Arg Leu Asp His Ala Phe Asp Lys Ala His
 210 215 220
 Thr Ser Leu Gly Ile Glu Lys Leu Leu Ser Pro Glu Thr Val Ala Val
 225 230 235 240
 His Leu Pro Asp Lys Lys Ser Ile Ile Met Tyr Leu Thr Ser Leu Phe
 245 250 255
 Glu Val Leu Pro Gln Gln Val Thr Ile Asp Ala Ile Arg Glu Val Glu
 260 265 270
 Thr Leu Pro Arg Lys Tyr Lys Lys Glu Cys Glu Glu Glu Glu Ile His
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 Ile Gln Ser Ala Val Leu Ala Glu Glu Gly Gln Ser Pro Arg Ala Glu
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 Thr Pro Ser Thr Val Thr Glu Val Asp Met Asp Leu Asp Ser Tyr Gln
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 Ile Ala Leu Glu Glu Val Leu Thr Trp Leu Leu Ser Ala Glu Asp Thr
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Glu Lys Ile Met Ala Phe Arg Glu Glu Ile Ala Pro Ile Asn Phe Lys
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 Val Lys Thr Val Asn Asp Leu Ser Ser Gln Leu Ser Pro Leu Asp Leu
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 His Pro Ser Leu Lys Met Ser Arg Gln Leu Asp Asp Leu Asn Met Arg
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 Trp Lys Leu Leu Gln Val Ser Val Asp Asp Arg Leu Lys Gln Leu Gln
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 Glu Ala His Arg Asp Phe Gly Pro Ser Ser Gln His Phe Leu Ser Thr
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 Cys Leu Asp Leu Leu Glu Leu Ser Thr Thr Asn Glu Ile Phe Lys Gln
 1460 1465 1470
 His Lys Leu Asn Gln Asn Asp Gln Leu Leu Ser Val Pro Asp Val Ile
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 1585 1590 1595 1600
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 Lys Cys Asn Ile Cys Lys Glu Cys Pro Ile Val Gly Phe Arg Tyr Arg
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 Ser Leu Lys His Phe Asn Tyr Asp Val Cys Gln Ser Cys Phe Phe Ser
 1665 1670 1675 1680
 Gly Arg Thr Ala Lys Gly His Lys Leu His Tyr Pro Met Val Glu Tyr
 1685 1690 1695
 Cys Ile Pro Thr Thr Ser Gly Glu Asp Val Arg Asp Phe Thr Lys Val
 1700 1705 1710
 Leu Lys Asn Lys Phe Arg Ser Lys Lys Tyr Phe Ala Lys His Pro Arg
 1715 1720 1725
 Leu Gly Tyr Leu Pro Val Gln Thr Val Leu Glu Gly Asp Asn Leu Glu
 1730 1735 1740
 Thr Pro Ile Thr Leu Ile Ser Met Trp Pro Glu His Tyr Asp Pro Ser
 1745 1750 1755 1760
 Gln Ser Pro Gln Leu Phe His Asp Asp Thr His Ser Arg Ile Glu Gln
 1765 1770 1775
 Tyr Ala Thr Arg Leu Ala Gln Met Glu Arg Thr Asn Gly Ser Phe Leu
 1780 1785 1790
 Thr Asp Ser Ser Ser Thr Thr Gly Ser Val Glu Asp Glu His Ala Leu

1795 1800 1805
 Ile Gln Gln Tyr Cys Gln Thr Leu Gly Gly Glu Ser Pro Val Ser Gln
 1810 1815 1820
 Pro Gln Ser Pro Ala Gln Ile Leu Lys Ser Val Glu Arg Glu Glu Arg
 1825 1830 1835 1840
 Gly Glu Leu Glu Arg Ile Ile Ala Asp Leu Glu Glu Gln Arg Asn
 1845 1850 1855
 Leu Gln Val Glu Tyr Glu Gln Leu Lys Asp Gln His Leu Arg Arg Gly
 1860 1865 1870
 Leu Pro Val Gly Ser Pro Pro Glu Ser Ile Ile Ser Pro His His Thr
 1875 1880 1885
 Ser Glu Asp Ser Glu Leu Ile Ala Glu Ala Lys Leu Leu Arg Gln His
 1890 1895 1900
 Lys Gly Arg Leu Glu Ala Arg Met Gln Ile Leu Glu Asp His Asn Lys
 1905 1910 1915 1920
 Gln Leu Glu Ser Gln Leu His Arg Leu Arg Gln Leu Leu Glu Gln Pro
 1925 1930 1935
 Glu Ser Asp Ser Arg Ile Asn Gly Val Ser Pro Trp Ala Ser Pro Gln
 1940 1945 1950
 His Ser Ala Leu Ser Tyr Ser Leu Asp Pro Asp Ala Ser Gly Pro Gln
 1955 1960 1965
 Phe His Gln Ala Ala Gly Glu Asp Leu Leu Ala Pro Pro His Asp Thr
 1970 1975 1980
 Ser Thr Asp Leu Thr Glu Val Met Glu Gln Ile His Ser Thr Phe Pro
 1985 1990 1995 2000
 Ser Cys Cys Pro Asn Val Pro Ser Arg Pro Gln Ala Met
 2005 2010

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 <213> Homo sapiens

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25

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 obtainable from non-human mammal

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22

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23

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21

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22

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<400> 20
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<210> 21
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<400> 22
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24

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22

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<400> 26

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22

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28

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28